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(54) Title: HUMAN HYDROLYTIC ENZYMES

(57) Abstract: The invention provides human hydrolytic enzymes (HYENZ.) and polynucleotides which identify and encode HYENZ. The invention also provides expression vectors, host cells, antibodies, agonists, and antagonists. The invention also provides methods for diagnosing, treating, or preventing disorders associated with expression of HYENZ.



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HUMAN HYDROLYTIC ENZYMES

TECHNICAL FIELD

This invention relates to nucleic acid and amino acid sequences of hydrolytic enzymes and to the use of these sequences in the diagnosis, treatment, and prevention of neurological disorders, immune system disorders, genetic disorders, and cell proliferation disorders, including cancer.

BACKGROUND OF THE INVENTION

Hydrolysis is the breaking of a covalent bond in a substrate by introduction of a water molecule. The reaction involves a nucleophilic attack by the water molecule's oxygen atom on a target bond in the substrate. The water molecule is split across the target bond, breaking the bond and generating two product molecules. Hydrolytic enzymes participate in reactions essential to functions such as cell signaling, cell proliferation, inflammation, apoptosis, secretion and excretion. Hydrolytic enzymes are involved in key steps in disease processes involving these functions. Hydrolytic enzymes, or hydrolases, may be grouped by substrate specificity into classes including aminohydrolases, phospholipases, carboxyl-esterases, phosphodiesterases, lysozymes, glycosidases, glyoxalases, sulfatases, phosphohydrolases, and serine hydrolases.

NG, NG-dimethylarginine dimethylaminohydrolase (DDAH) is an enzyme that hydrolyzes the endogenous nitric oxide synthase (NOS) inhibitors, NG-monomethyl-arginine and NG, NG-dimethyl-L-arginine to L-citrulline. Inhibiting DDAH can cause increased intracellular concentration of NOS inhibitors to levels sufficient to inhibit NOS. Therefore, DDAH inhibition may provide a method of NOS inhibition and changes in the activity of DDAH could play a role in pathophysiological alterations in nitric oxide generation (MacAllister, R.J., et al. (1996) Br. J. Pharmacol. 119: 1533-1540). DDAH was found in neurons displaying cytoskeletal abnormalities and oxidative stress in Alzheimer's disease. In age-matched control cases, DDAH was not found in neurons. This suggests that oxidative stress- and nitric oxide-mediated events play a role in the pathogenesis of Alzheimer's disease (Smith, M.A., et al. (1998) Free Radic. Biol. Med. 25: 898-902).

Dipeptidyl peptidase III is an enzyme that catalyzes the release of an N-terminal dipeptide from a peptide of four or more residues. It is localized to the cytosol and is active at neutral pH. It is inactive on Glu(4), Gly(4), and bonds involving proline. (See ExPasy - ENZYME, EC 3.4.14.4.)

Peptide deformylase hydrolyzes the formyl group at the N-terminus of newly synthesized polypeptides in prokaryotes. Deletion of the gene encoding peptide deformylase is lethal in *E. coli*. This lethality makes peptide deformylase a target for the design of new antibiotics (Becker, A. et al. (1998) J. Biol. Chem. 273:11413-11416 and Rajagopalan, P.T.R. and Pei, D. (1998) J. Biol. Chem. 273:22305-22310).

Trehalase is an enzyme that hydrolyzes trehalose, a protein that is thought to play a role in thermotolerance and dessication tolerance in yeast. Neutral trehalase is localized in the cytosol, while acid trehalase is localized in the vacuole. There is strong evidence that it is the neutral trehalase that hydrolyzes trehalose in intact cells. Evidence also suggests that the enhanced thermotolerance due to increased levels of trehalase is not due to the accumulation of trehalose. Trehalase may interact with heat shock protein 70 (Nwaka, S., et al. (1995) J. Biol. Chem. 270:10193-10198).

Phosphodiesterases catalyze the hydrolysis of one of the two ester bonds in a phosphodiester compound. Phosphodiesterases are, therefore, crucial to a variety of cellular processes. Phosphodiesterases include DNA and RNA endo- and exo-nucleases, which are essential to cell growth and replication as well as protein synthesis.

Pancreatic lipase and colipase form a complex that plays a key role in dietary fat digestion by converting insoluble long chain triacylglycerols into more polar molecules able to cross the brush border of intestinal cells. Colipase binds to the C-terminal domain of lipase. In solution, this interaction involves the formation of an ion pair between a glutamic acid residue of colipase and a lysine residue of lipase. These residues are strictly conserved among species (Ayvazian, L., et. al. (1998) J. Biol. Chem. 273(50): 33604-33609). Colipase appears to overcome the inhibitory effects of bile salts on pancreatic lipase (Online Mendelian Inheritance in Man (OMIM) 246600). Diacylglycerol lipase hydrolyzes triacylglycerol, diacylglycerol and other low-density lipoproteins (ExPASy - ENZYME, EC 3.1.1.34).

Carboxylesterases are proteins that hydrolyze carboxylic esters and are classified into three categories- A, B, and C. Most type-B carboxylesterases are evolutionarily related and are considered to comprise a family of proteins. The type-B carboxylesterase family of proteins includes vertebrate acetylcholinesterase, mammalian liver microsomal carboxylesterase, mammalian bile-salt-activated lipase, and duck fatty acyl-CoA hydrolase. Some members of this protein family are not catalytically active but contain a domain related evolutionarily to other type-B carboxylesterases, such as thyroglobulin and Drosophila protein neuractin. The active site of carboxylesterases involves three residues: a serine, a glutamate or aspartate, and a histidine. The sequence surrounding this catalytic site is well conserved and can be used as a signature pattern (PROSITE: PDOC00112).

Lysozyme c superfamily consists of conventional lysozymes c, calcium-binding lysozymes c, and α -lactalbumin (Prager, E.M. and Jolles, P. (1996) EXS 75: 9-31). The proteins in this superfamily have 35-40% sequence homology and share a common three dimensional fold, but can have different functions. Lysozymes bind and cleave the glycosidic bond linkage in sugars (Iyer, L.K. and Qasba, P.K. (1999) Protein Eng. 12: 129-139). Lysozymes c are ubiquitous in a variety of tissues and secretions and can lyse the cell walls of ceratin bacteria (McKenzie, H.A. (1996) EXS 75: 365-409). Alpha-lactalbumin is a metallo-protein that binds calcium and participates in the synthesis

of lactose (Iyer, L.K. and Qasba, P.K. (1999) Protein Eng. 12: 129-139). Alpha-lactalbumin occurs in mammalian milk and colostrum (McKenzie, supra).

The glyoxylase system consists of glyoxalase I, which catalyzes the formation of S-D-lactoylglutathione from methyglyoxal, a side product of triose-phosphate energy metabolism, and glyoxylase II, which hydrolyzes S-D-lactoylglutathione to D-lactic acid and reduced glutathione. Methyglyoxal levels are elevated during hyperglycemia, likely due to increased triose-phosphate energy metabolism. Elevated levels of glyoxylase II activity have been found in human and in a rat model of non-insulin-dependent diabetes mellitus. The glyoxylase system has been implicated in the detoxification of bacterial toxins, and in the control of cell proliferation and microtubule assembly. Elevated levels of S-D-lactoylglutathione, the substrate of glyoxylase II, induced growth arrest and toxicity in HL60 cells. Thus, the glyoxylase system, and glyoxylase II in particular, may be associated with cell proliferation and autoimmune system disorders such as diabetes.

Sulfatases are members of a highly conserved gene family that share extensive sequence homology and a high degree of structural similarity. Sulfatases catalyze the cleavage of sulfate esters. To perform this function, sulfatases undergo a unique posttranslational modification in the endoplasmic reticulum that involves the oxidation of a conserved cysteine residue. A human disorder called multiple sulfatase deficiency is due to a defect in this posttranslational modification step, leading to inactive sulfatases (Recksiek, M., et al. (1998) J. Biol. Chem. 273: 6096-6103).

Phosphohydrolases are enzymes that catalyze the hydrolysis of phosphate esters. Some phosphohydrolases contain a mutT domain signature sequence. MutT is a protein involved in the GO system responsible for removing an oxidatively damaged form of guanine from DNA. A region of about 40 amino acid residues, found in the N-terminus of mutT, is also found in other proteins, including some phosphohydrolases (PROSITE: PDOC00695).

Phosphatidic acid phosphohydrolases (PAPs) catalyze the dephosphorylation of phosphatidic acid to form diacylglycerol. The hydrolysis of phosphatidic acid by PAP terminates the signaling functions of phosphatidic acid and, by generating diacylglycerol, activates Ca^{2+} - and phospholipid-dependent protein kinase C enzymes. PAP-2 is localized to the plasma membrane and is independent of Mg^{2+} . It may play a role in modulating the signaling functions of phosphatidic acid, lysophosphatidic acid, and sphingomyelin-derived lipid phosphomonoesters. Three isozymes of PAP have been found in humans to date: PAP-2a, PAP-2b, and PAP-2c. (See, Roberts, R. et al. (1998) J. Biol. Chem. 273:22059-22067.)

Glycosidases catalyze the cleavage of hemiacetyl bonds of glycosides, which are compounds that contain one or more sugar. Mammalian beta-galactosidase removes the terminal galactose from gangliosides, glycoproteins, and glycosaminoglycans. Beta-galactosidases belong to family 35 in the classification of glycosyl hydrolases. Deficiency of this enzyme is associated with the genetic disease

GM1-gangliosidosis known as Morquio disease type B (PROSITE: PDOC00910).

Serine hydrolases are a functional class of hydrolytic enzymes that contain a serine residue in their active site. This class of enzymes contains proteinases, esterases, and lipases which hydrolyze a variety of substrates and, therefore, have different biological roles. Proteins in this superfamily can be further grouped into subfamilies based on substrate specificity or amino acid similarities (Puente, X.S. and Lopez-Ont, C. (1995) J. Biol. Chem. 270: 12926-12932).

The discovery of new hydrolytic enzymes and the polynucleotides encoding them satisfies a need in the art by providing new compositions which are useful in the diagnosis, prevention, and treatment of neurological disorders, immune system disorders, genetic disorders, and cell proliferation disorders, including cancer.

SUMMARY OF THE INVENTION

The invention features purified polypeptides, hydrolytic enzymes, referred to collectively as "HYENZ" and individually as "HYENZ-1," "HYENZ-2," "HYENZ-3," "HYENZ-4," "HYENZ-5," "HYENZ-6," "HYENZ-7," "HYENZ-8," "HYENZ-9," "HYENZ-10," "HYENZ-11," "HYENZ-12," "HYENZ-13," and "HYENZ-14." In one aspect, the invention provides an isolated polypeptide comprising an amino acid sequence selected from the group consisting of a) an amino acid sequence selected from the group consisting of SEQ ID NO:1-14, b) a naturally occurring amino acid sequence having at least 90% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO:1-14, c) a biologically active fragment of an amino acid sequence selected from the group consisting of SEQ ID NO:1-14, and d) an immunogenic fragment of an amino acid sequence selected from the group consisting of SEQ ID NO:1-14. In one alternative, the invention provides an isolated polypeptide comprising the amino acid sequence of SEQ ID NO:1-14.

The invention further provides an isolated polynucleotide encoding a polypeptide comprising an amino acid sequence selected from the group consisting of a) an amino acid sequence selected from the group consisting of SEQ ID NO:1-14, b) a naturally occurring amino acid sequence having at least 90% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO:1-14, c) a biologically active fragment of an amino acid sequence selected from the group consisting of SEQ ID NO:1-14, and d) an immunogenic fragment of an amino acid sequence selected from the group consisting of SEQ ID NO:1-14. In one alternative, the polynucleotide encodes a polypeptide selected from the group consisting of SEQ ID NO:1-14. In another alternative, the polynucleotide is selected from the group consisting of SEQ ID NO:15-28.

Additionally, the invention provides a recombinant polynucleotide comprising a promoter sequence operably linked to a polynucleotide encoding a polypeptide comprising an amino acid sequence selected from the group consisting of a) an amino acid sequence selected from the group

consisting of SEQ ID NO:1-14, b) a naturally occurring amino acid sequence having at least 90% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO:1-14, c) a biologically active fragment of an amino acid sequence selected from the group consisting of SEQ ID NO:1-14, and d) an immunogenic fragment of an amino acid sequence selected from the group consisting of SEQ ID NO:1-14. In one alternative, the invention provides a cell transformed with the recombinant polynucleotide. In another alternative, the invention provides a transgenic organism comprising the recombinant polynucleotide.

The invention also provides a method for producing a polypeptide comprising an amino acid sequence selected from the group consisting of a) an amino acid sequence selected from the group consisting of SEQ ID NO:1-14, b) a naturally occurring amino acid sequence having at least 90% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO:1-14, c) a biologically active fragment of an amino acid sequence selected from the group consisting of SEQ ID NO:1-14, and d) an immunogenic fragment of an amino acid sequence selected from the group consisting of SEQ ID NO:1-14. The method comprises a) culturing a cell under conditions suitable for expression of the polypeptide, wherein said cell is transformed with a recombinant polynucleotide comprising a promoter sequence operably linked to a polynucleotide encoding the polypeptide, and b) recovering the polypeptide so expressed.

Additionally, the invention provides an isolated antibody which specifically binds to a polypeptide comprising an amino acid sequence selected from the group consisting of a) an amino acid sequence selected from the group consisting of SEQ ID NO:1-14, b) a naturally occurring amino acid sequence having at least 90% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO:1-14, c) a biologically active fragment of an amino acid sequence selected from the group consisting of SEQ ID NO:1-14, and d) an immunogenic fragment of an amino acid sequence selected from the group consisting of SEQ ID NO:1-14.

The invention further provides an isolated polynucleotide comprising a polynucleotide sequence selected from the group consisting of a) a polynucleotide sequence selected from the group consisting of SEQ ID NO:15-28, b) a naturally occurring polynucleotide sequence having at least 70% sequence identity to a polynucleotide sequence selected from the group consisting of SEQ ID NO:15-28, c) a polynucleotide sequence complementary to a), d) a polynucleotide sequence complementary to b), and e) an RNA equivalent of a)-d). In one alternative, the polynucleotide comprises at least 60 contiguous nucleotides.

Additionally, the invention provides a method for detecting a target polynucleotide in a sample, said target polynucleotide having a sequence of a polynucleotide comprising a polynucleotide sequence selected from the group consisting of a) a polynucleotide sequence selected from the group consisting of

SEQ ID NO:15-28, b) a naturally occurring polynucleotide sequence having at least 70% sequence identity to a polynucleotide sequence selected from the group consisting of SEQ ID NO:15-28, c) a polynucleotide sequence complementary to a), d) a polynucleotide sequence complementary to b), and e) an RNA equivalent of a)-d). The method comprises a) hybridizing the sample with a probe comprising at least 20 contiguous nucleotides comprising a sequence complementary to said target polynucleotide in the sample, and which probe specifically hybridizes to said target polynucleotide, under conditions whereby a hybridization complex is formed between said probe and said target polynucleotide or fragments thereof, and b) detecting the presence or absence of said hybridization complex, and optionally, if present, the amount thereof. In one alternative, the probe comprises at least 60 contiguous nucleotides.

The invention further provides a method for detecting a target polynucleotide in a sample, said target polynucleotide having a sequence of a polynucleotide comprising a polynucleotide sequence selected from the group consisting of a) a polynucleotide sequence selected from the group consisting of SEQ ID NO:15-28, b) a naturally occurring polynucleotide sequence having at least 70% sequence identity to a polynucleotide sequence selected from the group consisting of SEQ ID NO:15-28, c) a polynucleotide sequence complementary to a), d) a polynucleotide sequence complementary to b), and e) an RNA equivalent of a)-d). The method comprises a) amplifying said target polynucleotide or fragment thereof using polymerase chain reaction amplification, and b) detecting the presence or absence of said amplified target polynucleotide or fragment thereof, and, optionally, if present, the amount thereof.

The invention further provides a composition comprising an effective amount of a polypeptide comprising an amino acid sequence selected from the group consisting of a) an amino acid sequence selected from the group consisting of SEQ ID NO:1-14, b) a naturally occurring amino acid sequence having at least 90% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO:1-14, c) a biologically active fragment of an amino acid sequence selected from the group consisting of SEQ ID NO:1-14, and d) an immunogenic fragment of an amino acid sequence selected from the group consisting of SEQ ID NO:1-14, and a pharmaceutically acceptable excipient. In one embodiment, the composition comprises an amino acid sequence selected from the group consisting of SEQ ID NO:1-14. The invention additionally provides a method of treating a disease or condition associated with decreased expression of functional HYENZ, comprising administering to a patient in need of such treatment the composition.

The invention also provides a method for screening a compound for effectiveness as an agonist of a polypeptide comprising an amino acid sequence selected from the group consisting of a) an amino acid sequence selected from the group consisting of SEQ ID NO:1-14, b) a naturally occurring

amino acid sequence having at least 90% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO:1-14, c) a biologically active fragment of an amino acid sequence selected from the group consisting of SEQ ID NO:1-14, and d) an immunogenic fragment of an amino acid sequence selected from the group consisting of SEQ ID NO:1-14. The method comprises a) exposing a sample comprising the polypeptide to a compound, and b) detecting agonist activity in the sample. In one alternative, the invention provides a composition comprising an agonist compound identified by the method and a pharmaceutically acceptable excipient. In another alternative, the invention provides a method of treating a disease or condition associated with decreased expression of functional HYENZ, comprising administering to a patient in need of such treatment the composition.

Additionally, the invention provides a method for screening a compound for effectiveness as an antagonist of a polypeptide comprising an amino acid sequence selected from the group consisting of a) an amino acid sequence selected from the group consisting of SEQ ID NO:1-14, b) a naturally occurring amino acid sequence having at least 90% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO:1-14, c) a biologically active fragment of an amino acid sequence selected from the group consisting of SEQ ID NO:1-14, and d) an immunogenic fragment of an amino acid sequence selected from the group consisting of SEQ ID NO:1-14. The method comprises a) exposing a sample comprising the polypeptide to a compound, and b) detecting antagonist activity in the sample. In one alternative, the invention provides a composition comprising an antagonist compound identified by the method and a pharmaceutically acceptable excipient. In another alternative, the invention provides a method of treating a disease or condition associated with overexpression of functional HYENZ, comprising administering to a patient in need of such treatment the composition.

The invention further provides a method of screening for a compound that specifically binds to a polypeptide comprising an amino acid sequence selected from the group consisting of a) an amino acid sequence selected from the group consisting of SEQ ID NO:1-14, b) a naturally occurring amino acid sequence having at least 90% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO:1-14, c) a biologically active fragment of an amino acid sequence selected from the group consisting of SEQ ID NO:1-14, and d) an immunogenic fragment of an amino acid sequence selected from the group consisting of SEQ ID NO:1-14. The method comprises a) combining the polypeptide with at least one test compound under suitable conditions, and b) detecting binding of the polypeptide to the test compound, thereby identifying a compound that specifically binds to the polypeptide.

The invention further provides a method of screening for a compound that modulates the activity of a polypeptide comprising an amino acid sequence selected from the group consisting of a) an amino acid sequence selected from the group consisting of SEQ ID NO:1-14, b) a naturally

occurring amino acid sequence having at least 90% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO:1-14, c) a biologically active fragment of an amino acid sequence selected from the group consisting of SEQ ID NO:1-14, and d) an immunogenic fragment of an amino acid sequence selected from the group consisting of SEQ ID NO:1-14. The

5 method comprises a) combining the polypeptide with at least one test compound under conditions permissive for the activity of the polypeptide, b) assessing the activity of the polypeptide in the presence of the test compound, and c) comparing the activity of the polypeptide in the presence of the test compound with the activity of the polypeptide in the absence of the test compound, wherein a change in the activity of the polypeptide in the presence of the test compound is indicative of a
10 compound that modulates the activity of the polypeptide.

The invention further provides a method for screening a compound for effectiveness in altering expression of a target polynucleotide, wherein said target polynucleotide comprises a sequence selected from the group consisting of SEQ ID NO:15-28, the method comprising a) exposing a sample comprising the target polynucleotide to a compound, and b) detecting altered
15 expression of the target polynucleotide.

The invention further provides a method for assessing toxicity of a test compound, said method comprising a) treating a biological sample containing nucleic acids with the test compound; b) hybridizing the nucleic acids of the treated biological sample with a probe comprising at least 20 contiguous nucleotides of a polynucleotide comprising a polynucleotide sequence selected from the
20 group consisting of i) a polynucleotide sequence selected from the group consisting of SEQ ID NO:15-28, ii) a naturally occurring polynucleotide sequence having at least 70% sequence identity to a polynucleotide sequence selected from the group consisting of SEQ ID NO:15-28, iii) a polynucleotide sequence complementary to i), iv) a polynucleotide sequence complementary to ii), and v) an RNA equivalent of i)-iv). Hybridization occurs under conditions whereby a specific
25 hybridization complex is formed between said probe and a target polynucleotide in the biological sample, said target polynucleotide comprising a polynucleotide sequence selected from the group consisting of i) a polynucleotide sequence selected from the group consisting of SEQ ID NO:15-28, ii) a naturally occurring polynucleotide sequence having at least 70% sequence identity to a polynucleotide sequence selected from the group consisting of SEQ ID NO:15-28, iii) a
30 polynucleotide sequence complementary to i), iv) a polynucleotide sequence complementary to ii), and v) an RNA equivalent of i)-iv). Alternatively, the target polynucleotide comprises a fragment of a polynucleotide sequence selected from the group consisting of i)-v) above; c) quantifying the amount of hybridization complex; and d) comparing the amount of hybridization complex in the treated biological sample with the amount of hybridization complex in an untreated biological
35 sample, wherein a difference in the amount of hybridization complex in the treated biological sample

is indicative of toxicity of the test compound.

BRIEF DESCRIPTION OF THE TABLES

Table 1 shows polypeptide and nucleotide sequence identification numbers (SEQ ID NOs),
5 clone identification numbers (clone IDs), cDNA libraries, and cDNA fragments used to assemble full-length sequences encoding HYENZ.

Table 2 shows features of each polypeptide sequence, including potential motifs, homologous sequences, and methods, algorithms, and searchable databases used for analysis of HYENZ.

Table 3 shows selected fragments of each nucleic acid sequence; the tissue-specific expression
10 patterns of each nucleic acid sequence as determined by northern analysis; diseases, disorders, or conditions associated with these tissues; and the vector into which each cDNA was cloned.

Table 4 describes the tissues used to construct the cDNA libraries from which cDNA clones encoding HYENZ were isolated.

Table 5 shows the tools, programs, and algorithms used to analyze the polynucleotides and
15 polypeptides of the invention, along with applicable descriptions, references, and threshold parameters.

DESCRIPTION OF THE INVENTION

Before the present proteins, nucleotide sequences, and methods are described, it is understood that this invention is not limited to the particular machines, materials and methods described, as these
20 may vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to limit the scope of the present invention which will be limited only by the appended claims.

It must be noted that as used herein and in the appended claims, the singular forms "a," "an," and "the" include plural reference unless the context clearly dictates otherwise. Thus, for example, a
25 reference to "a host cell" includes a plurality of such host cells, and a reference to "an antibody" is a reference to one or more antibodies and equivalents thereof known to those skilled in the art, and so forth.

Unless defined otherwise, all technical and scientific terms used herein have the same meanings as commonly understood by one of ordinary skill in the art to which this invention belongs. Although
30 any machines, materials, and methods similar or equivalent to those described herein can be used to practice or test the present invention, the preferred machines, materials and methods are now described. All publications mentioned herein are cited for the purpose of describing and disclosing the cell lines, protocols, reagents and vectors which are reported in the publications and which might be used in connection with the invention. Nothing herein is to be construed as an admission that the invention is

not entitled to antedate such disclosure by virtue of prior invention.

DEFINITIONS

"HYENZ" refers to the amino acid sequences of substantially purified HYENZ obtained from any species, particularly a mammalian species, including bovine, ovine, porcine, murine, equine, and human, and from any source, whether natural, synthetic, semi-synthetic, or recombinant.

The term "agonist" refers to a molecule which intensifies or mimics the biological activity of HYENZ. Agonists may include proteins, nucleic acids, carbohydrates, small molecules, or any other compound or composition which modulates the activity of HYENZ either by directly interacting with HYENZ or by acting on components of the biological pathway in which HYENZ participates.

An "allelic variant" is an alternative form of the gene encoding HYENZ. Allelic variants may result from at least one mutation in the nucleic acid sequence and may result in altered mRNAs or in polypeptides whose structure or function may or may not be altered. A gene may have none, one, or many allelic variants of its naturally occurring form. Common mutational changes which give rise to allelic variants are generally ascribed to natural deletions, additions, or substitutions of nucleotides. Each of these types of changes may occur alone, or in combination with the others, one or more times in a given sequence.

"Altered" nucleic acid sequences encoding HYENZ include those sequences with deletions, insertions, or substitutions of different nucleotides, resulting in a polypeptide the same as HYENZ or a polypeptide with at least one functional characteristic of HYENZ. Included within this definition are polymorphisms which may or may not be readily detectable using a particular oligonucleotide probe of the polynucleotide encoding HYENZ, and improper or unexpected hybridization to allelic variants, with a locus other than the normal chromosomal locus for the polynucleotide sequence encoding HYENZ. The encoded protein may also be "altered," and may contain deletions, insertions, or substitutions of amino acid residues which produce a silent change and result in a functionally equivalent HYENZ.

Deliberate amino acid substitutions may be made on the basis of similarity in polarity, charge, solubility, hydrophobicity, hydrophilicity, and/or the amphipathic nature of the residues, as long as the biological or immunological activity of HYENZ is retained. For example, negatively charged amino acids may include aspartic acid and glutamic acid, and positively charged amino acids may include lysine and arginine. Amino acids with uncharged polar side chains having similar hydrophilicity values may include: asparagine and glutamine; and serine and threonine. Amino acids with uncharged side chains having similar hydrophilicity values may include: leucine, isoleucine, and valine; glycine and alanine; and phenylalanine and tyrosine.

The terms "amino acid" and "amino acid sequence" refer to an oligopeptide, peptide, polypeptide, or protein sequence, or a fragment of any of these, and to naturally occurring or synthetic molecules. Where "amino acid sequence" is recited to refer to a sequence of a naturally occurring

protein molecule, "amino acid sequence" and like terms are not meant to limit the amino acid sequence to the complete native amino acid sequence associated with the recited protein molecule.

"Amplification" relates to the production of additional copies of a nucleic acid sequence.

Amplification is generally carried out using polymerase chain reaction (PCR) technologies well known in the art.

The term "antagonist" refers to a molecule which inhibits or attenuates the biological activity of HYENZ. Antagonists may include proteins such as antibodies, nucleic acids, carbohydrates, small molecules, or any other compound or composition which modulates the activity of HYENZ either by directly interacting with HYENZ or by acting on components of the biological pathway in which HYENZ participates.

The term "antibody" refers to intact immunoglobulin molecules as well as to fragments thereof, such as Fab, F(ab')₂, and Fv fragments, which are capable of binding an epitopic determinant. Antibodies that bind HYENZ polypeptides can be prepared using intact polypeptides or using fragments containing small peptides of interest as the immunizing antigen. The polypeptide or oligopeptide used to immunize an animal (e.g., a mouse, a rat, or a rabbit) can be derived from the translation of RNA, or synthesized chemically, and can be conjugated to a carrier protein if desired. Commonly used carriers that are chemically coupled to peptides include bovine serum albumin, thyroglobulin, and keyhole limpet hemocyanin (KLH). The coupled peptide is then used to immunize the animal.

The term "antigenic determinant" refers to that region of a molecule (i.e., an epitope) that makes contact with a particular antibody. When a protein or a fragment of a protein is used to immunize a host animal, numerous regions of the protein may induce the production of antibodies which bind specifically to antigenic determinants (particular regions or three-dimensional structures on the protein). An antigenic determinant may compete with the intact antigen (i.e., the immunogen used to elicit the immune response) for binding to an antibody.

The term "antisense" refers to any composition capable of base-pairing with the "sense" (coding) strand of a specific nucleic acid sequence. Antisense compositions may include DNA; RNA; peptide nucleic acid (PNA); oligonucleotides having modified backbone linkages such as phosphorothioates, methylphosphonates, or benzylphosphonates; oligonucleotides having modified sugar groups such as 2'-methoxyethyl sugars or 2'-methoxyethoxy sugars; or oligonucleotides having modified bases such as 5-methyl cytosine, 2'-deoxyuracil, or 7-deaza-2'-deoxyguanosine. Antisense molecules may be produced by any method including chemical synthesis or transcription. Once introduced into a cell, the complementary antisense molecule base-pairs with a naturally occurring nucleic acid sequence produced by the cell to form duplexes which block either transcription or

translation. The designation "negative" or "minus" can refer to the antisense strand, and the designation "positive" or "plus" can refer to the sense strand of a reference DNA molecule.

The term "biologically active" refers to a protein having structural, regulatory, or biochemical functions of a naturally occurring molecule. Likewise, "immunologically active" or "immunogenic" refers to the capability of the natural, recombinant, or synthetic HYENZ, or of any oligopeptide thereof, to induce a specific immune response in appropriate animals or cells and to bind with specific antibodies.

"Complementary" describes the relationship between two single-stranded nucleic acid sequences that anneal by base-pairing. For example, 5'-AGT-3' pairs with its complement, 3'-TCA-5'.

A "composition comprising a given polynucleotide sequence" and a "composition comprising a given amino acid sequence" refer broadly to any composition containing the given polynucleotide or amino acid sequence. The composition may comprise a dry formulation or an aqueous solution. Compositions comprising polynucleotide sequences encoding HYENZ or fragments of HYENZ may be employed as hybridization probes. The probes may be stored in freeze-dried form and may be associated with a stabilizing agent such as a carbohydrate. In hybridizations, the probe may be deployed in an aqueous solution containing salts (e.g., NaCl), detergents (e.g., sodium dodecyl sulfate; SDS), and other components (e.g., Denhardt's solution, dry milk, salmon sperm DNA, etc.).

"Consensus sequence" refers to a nucleic acid sequence which has been subjected to repeated DNA sequence analysis to resolve uncalled bases, extended using the XL-PCR kit (PE Biosystems, Foster City CA) in the 5' and/or the 3' direction, and resequenced, or which has been assembled from one or more overlapping cDNA, EST, or genomic DNA fragments using a computer program for fragment assembly, such as the GELVIEW fragment assembly system (GCG, Madison WI) or Phrap (University of Washington, Seattle WA). Some sequences have been both extended and assembled to produce the consensus sequence.

"Conservative amino acid substitutions" are those substitutions that are predicted to least interfere with the properties of the original protein, i.e., the structure and especially the function of the protein is conserved and not significantly changed by such substitutions. The table below shows amino acids which may be substituted for an original amino acid in a protein and which are regarded as conservative amino acid substitutions.

Original Residue	Conservative Substitution
Ala	Gly, Ser
Arg	His, Lys
Asn	Asp, Gln, His
Asp	Asn, Glu
Cys	Ala, Ser

	Gln	Asn, Glu, His
	Glu	Asp, Gln, His
	Gly	Ala
5	His	Asn, Arg, Gln, Glu
	Ile	Leu, Val
	Leu	Ile, Val
	Lys	Arg, Gln, Glu
	Met	Leu, Ile
10	Phe	His, Met, Leu, Trp, Tyr
	Ser	Cys, Thr
	Thr	Ser, Val
	Trp	Phe, Tyr
	Tyr	His, Phe, Trp
15	Val	Ile, Leu, Thr

Conservative amino acid substitutions generally maintain (a) the structure of the polypeptide backbone in the area of the substitution, for example, as a beta sheet or alpha helical conformation, (b) the charge or hydrophobicity of the molecule at the site of the substitution, and/or (c) the bulk of the side chain.

20 A "deletion" refers to a change in the amino acid or nucleotide sequence that results in the absence of one or more amino acid residues or nucleotides.

The term "derivative" refers to a chemically modified polynucleotide or polypeptide. Chemical modifications of a polynucleotide sequence can include, for example, replacement of hydrogen by an alkyl, acyl, hydroxyl, or amino group. A derivative polynucleotide encodes a polypeptide which retains
25 at least one biological or immunological function of the natural molecule. A derivative polypeptide is one modified by glycosylation, pegylation, or any similar process that retains at least one biological or immunological function of the polypeptide from which it was derived.

A "detectable label" refers to a reporter molecule or enzyme that is capable of generating a measurable signal and is covalently or noncovalently joined to a polynucleotide or polypeptide.

30 A "fragment" is a unique portion of HYENZ or the polynucleotide encoding HYENZ which is identical in sequence to but shorter in length than the parent sequence. A fragment may comprise up to the entire length of the defined sequence, minus one nucleotide/amino acid residue. For example, a fragment may comprise from 5 to 1000 contiguous nucleotides or amino acid residues. A fragment used as a probe, primer, antigen, therapeutic molecule, or for other purposes, may be at least
35 5, 10, 15, 16, 20, 25, 30, 40, 50, 60, 75, 100, 150, 250 or at least 500 contiguous nucleotides or amino acid residues in length. Fragments may be preferentially selected from certain regions of a molecule. For example, a polypeptide fragment may comprise a certain length of contiguous amino acids selected from the first 250 or 500 amino acids (or first 25% or 50% of a polypeptide) as shown in a certain defined sequence. Clearly these lengths are exemplary, and any length that is supported

by the specification, including the Sequence Listing, tables, and figures, may be encompassed by the present embodiments.

A fragment of SEQ ID NO:15-28 comprises a region of unique polynucleotide sequence that specifically identifies SEQ ID NO:15-28, for example, as distinct from any other sequence in the genome from which the fragment was obtained. A fragment of SEQ ID NO:15-28 is useful, for example, in hybridization and amplification technologies and in analogous methods that distinguish SEQ ID NO:15-28 from related polynucleotide sequences. The precise length of a fragment of SEQ ID NO:15-28 and the region of SEQ ID NO:15-28 to which the fragment corresponds are routinely determinable by one of ordinary skill in the art based on the intended purpose for the fragment.

A fragment of SEQ ID NO:1-14 is encoded by a fragment of SEQ ID NO:15-28. A fragment of SEQ ID NO:1-14 comprises a region of unique amino acid sequence that specifically identifies SEQ ID NO:1-14. For example, a fragment of SEQ ID NO:1-14 is useful as an immunogenic peptide for the development of antibodies that specifically recognize SEQ ID NO:1-14. The precise length of a fragment of SEQ ID NO:1-14 and the region of SEQ ID NO:1-14 to which the fragment corresponds are routinely determinable by one of ordinary skill in the art based on the intended purpose for the fragment.

A "full-length" polynucleotide sequence is one containing at least a translation initiation codon (e.g., methionine) followed by an open reading frame and a translation termination codon. A "full-length" polynucleotide sequence encodes a "full-length" polypeptide sequence.

"Homology" refers to sequence similarity or, interchangeably, sequence identity, between two or more polynucleotide sequences or two or more polypeptide sequences.

The terms "percent identity" and "% identity," as applied to polynucleotide sequences, refer to the percentage of residue matches between at least two polynucleotide sequences aligned using a standardized algorithm. Such an algorithm may insert, in a standardized and reproducible way, gaps in the sequences being compared in order to optimize alignment between two sequences, and therefore achieve a more meaningful comparison of the two sequences.

Percent identity between polynucleotide sequences may be determined using the default parameters of the CLUSTAL V algorithm as incorporated into the MEGALIGN version 3.12e sequence alignment program. This program is part of the LASERGENE software package, a suite of molecular biological analysis programs (DNASTAR, Madison WI). CLUSTAL V is described in Higgins, D.G. and P.M. Sharp (1989) CABIOS 5:151-153 and in Higgins, D.G. et al. (1992) CABIOS 8:189-191. For pairwise alignments of polynucleotide sequences, the default parameters are set as follows: Ktuple=2, gap penalty=5, window=4, and "diagonals saved"=4. The "weighted" residue weight table is selected as the default. Percent identity is reported by CLUSTAL V as the "percent similarity" between aligned polynucleotide sequences.

Alternatively, a suite of commonly used and freely available sequence comparison algorithms is provided by the National Center for Biotechnology Information (NCBI) Basic Local Alignment Search Tool (BLAST) (Altschul, S.F. et al. (1990) J. Mol. Biol. 215:403-410), which is available from several sources, including the NCBI, Bethesda, MD, and on the Internet at

5 <http://www.ncbi.nlm.nih.gov/BLAST/>. The BLAST software suite includes various sequence analysis programs including "blastn," that is used to align a known polynucleotide sequence with other polynucleotide sequences from a variety of databases. Also available is a tool called "BLAST 2 Sequences" that is used for direct pairwise comparison of two nucleotide sequences. "BLAST 2 Sequences" can be accessed and used interactively at <http://www.ncbi.nlm.nih.gov/gorf/bl2.html>. The
10 "BLAST 2 Sequences" tool can be used for both blastn and blastp (discussed below). BLAST programs are commonly used with gap and other parameters set to default settings. For example, to compare two nucleotide sequences, one may use blastn with the "BLAST 2 Sequences" tool Version 2.0.12 (April-21-2000) set at default parameters. Such default parameters may be, for example:

Matrix: BLOSUM62

15 *Reward for match: 1*

Penalty for mismatch: -2

Open Gap: 5 and Extension Gap: 2 penalties

Gap x drop-off: 50

Expect: 10

20 *Word Size: 11*

Filter: on

Percent identity may be measured over the length of an entire defined sequence, for example, as defined by a particular SEQ ID number, or may be measured over a shorter length, for example, over the length of a fragment taken from a larger, defined sequence, for instance, a fragment of at least 20, at
25 least 30, at least 40, at least 50, at least 70, at least 100, or at least 200 contiguous nucleotides. Such lengths are exemplary only, and it is understood that any fragment length supported by the sequences shown herein, in the tables, figures, or Sequence Listing, may be used to describe a length over which percentage identity may be measured.

Nucleic acid sequences that do not show a high degree of identity may nevertheless encode
30 similar amino acid sequences due to the degeneracy of the genetic code. It is understood that changes in a nucleic acid sequence can be made using this degeneracy to produce multiple nucleic acid sequences that all encode substantially the same protein.

The phrases "percent identity" and "% identity," as applied to polypeptide sequences, refer to the percentage of residue matches between at least two polypeptide sequences aligned using a

standardized algorithm. Methods of polypeptide sequence alignment are well-known. Some alignment methods take into account conservative amino acid substitutions. Such conservative substitutions, explained in more detail above, generally preserve the charge and hydrophobicity at the site of substitution, thus preserving the structure (and therefore function) of the polypeptide.

5 Percent identity between polypeptide sequences may be determined using the default parameters of the CLUSTAL V algorithm as incorporated into the MEGALIGN version 3.12e sequence alignment program (described and referenced above). For pairwise alignments of polypeptide sequences using CLUSTAL V, the default parameters are set as follows: Ktuple=1, gap penalty=3, window=5, and "diagonals saved"=5. The PAM250 matrix is selected as the default residue weight table. As with
10 polynucleotide alignments, the percent identity is reported by CLUSTAL V as the "percent similarity" between aligned polypeptide sequence pairs.

Alternatively the NCBI BLAST software suite may be used. For example, for a pairwise comparison of two polypeptide sequences, one may use the "BLAST 2 Sequences" tool Version 2.0.12 (Apr-21-2000) with blastp set at default parameters. Such default parameters may be, for example:

15 *Matrix: BLOSUM62*
 Open Gap: 11 and Extension Gap: 1 penalties
 Gap x drop-off: 50
 Expect: 10
 Word Size: 3
20 *Filter: on*

Percent identity may be measured over the length of an entire defined polypeptide sequence, for example, as defined by a particular SEQ ID number, or may be measured over a shorter length, for example, over the length of a fragment taken from a larger, defined polypeptide sequence, for instance, a fragment of at least 15, at least 20, at least 30, at least 40, at least 50, at least 70 or at least 150
25 contiguous residues. Such lengths are exemplary only, and it is understood that any fragment length supported by the sequences shown herein, in the tables, figures or Sequence Listing, may be used to describe a length over which percentage identity may be measured.

"Human artificial chromosomes" (HACs) are linear microchromosomes which may contain DNA sequences of about 6 kb to 10 Mb in size, and which contain all of the elements required for
30 chromosome replication, segregation and maintenance.

The term "humanized antibody" refers to an antibody molecule in which the amino acid sequence in the non-antigen binding regions has been altered so that the antibody more closely resembles a human antibody, and still retains its original binding ability.

"Hybridization" refers to the process by which a polynucleotide strand anneals with a

complementary strand through base pairing under defined hybridization conditions. Specific hybridization is an indication that two nucleic acid sequences share a high degree of complementarity. Specific hybridization complexes form under permissive annealing conditions and remain hybridized after the "washing" step(s). The washing step(s) is particularly important in determining the stringency of the hybridization process, with more stringent conditions allowing less non-specific binding, i.e., binding between pairs of nucleic acid strands that are not perfectly matched. Permissive conditions for annealing of nucleic acid sequences are routinely determinable by one of ordinary skill in the art and may be consistent among hybridization experiments, whereas wash conditions may be varied among experiments to achieve the desired stringency, and therefore hybridization specificity. Permissive annealing conditions occur, for example, at 68°C in the presence of about 6 x SSC, about 1% (w/v) SDS, and about 100 µg/ml sheared, denatured salmon sperm DNA.

Generally, stringency of hybridization is expressed, in part, with reference to the temperature under which the wash step is carried out. Such wash temperatures are typically selected to be about 5°C to 20°C lower than the thermal melting point (T_m) for the specific sequence at a defined ionic strength and pH. The T_m is the temperature (under defined ionic strength and pH) at which 50% of the target sequence hybridizes to a perfectly matched probe. An equation for calculating T_m and conditions for nucleic acid hybridization are well known and can be found in Sambrook, J. et al., 1989, Molecular Cloning: A Laboratory Manual, 2nd ed., vol. 1-3, Cold Spring Harbor Press, Plainview NY; specifically see volume 2, chapter 9.

High stringency conditions for hybridization between polynucleotides of the present invention include wash conditions of 68°C in the presence of about 0.2 x SSC and about 0.1% SDS, for 1 hour. Alternatively, temperatures of about 65°C, 60°C, 55°C, or 42°C may be used. SSC concentration may be varied from about 0.1 to 2 x SSC, with SDS being present at about 0.1%. Typically, blocking reagents are used to block non-specific hybridization. Such blocking reagents include, for instance, sheared and denatured salmon sperm DNA at about 100-200 µg/ml. Organic solvent, such as formamide at a concentration of about 35-50% v/v, may also be used under particular circumstances, such as for RNA:DNA hybridizations. Useful variations on these wash conditions will be readily apparent to those of ordinary skill in the art. Hybridization, particularly under high stringency conditions, may be suggestive of evolutionary similarity between the nucleotides. Such similarity is strongly indicative of a similar role for the nucleotides and their encoded polypeptides.

The term "hybridization complex" refers to a complex formed between two nucleic acid sequences by virtue of the formation of hydrogen bonds between complementary bases. A hybridization complex may be formed in solution (e.g., C_{ot} or R_{ot} analysis) or formed between one nucleic acid sequence present in solution and another nucleic acid sequence immobilized on a solid support (e.g.,

paper, membranes, filters, chips, pins or glass slides, or any other appropriate substrate to which cells or their nucleic acids have been fixed).

The words "insertion" and "addition" refer to changes in an amino acid or nucleotide sequence resulting in the addition of one or more amino acid residues or nucleotides, respectively.

5 "Immune response" can refer to conditions associated with inflammation, trauma, immune disorders, or infectious or genetic disease, etc. These conditions can be characterized by expression of various factors, e.g., cytokines, chemokines, and other signaling molecules, which may affect cellular and systemic defense systems.

10 An "immunogenic fragment" is a polypeptide or oligopeptide fragment of HYENZ which is capable of eliciting an immune response when introduced into a living organism, for example, a mammal. The term "immunogenic fragment" also includes any polypeptide or oligopeptide fragment of HYENZ which is useful in any of the antibody production methods disclosed herein or known in the art.

The term "microarray" refers to an arrangement of a plurality of polynucleotides, polypeptides, or other chemical compounds on a substrate.

15 The terms "element" and "array element" refer to a polynucleotide, polypeptide, or other chemical compound having a unique and defined position on a microarray.

The term "modulate" refers to a change in the activity of HYENZ. For example, modulation may cause an increase or a decrease in protein activity, binding characteristics, or any other biological, functional, or immunological properties of HYENZ.

20 The phrases "nucleic acid" and "nucleic acid sequence" refer to a nucleotide, oligonucleotide, polynucleotide, or any fragment thereof. These phrases also refer to DNA or RNA of genomic or synthetic origin which may be single-stranded or double-stranded and may represent the sense or the antisense strand, to peptide nucleic acid (PNA), or to any DNA-like or RNA-like material.

"Operably linked" refers to the situation in which a first nucleic acid sequence is placed in a
25 functional relationship with a second nucleic acid sequence. For instance, a promoter is operably linked to a coding sequence if the promoter affects the transcription or expression of the coding sequence. Operably linked DNA sequences may be in close proximity or contiguous and, where necessary to join two protein coding regions, in the same reading frame.

"Peptide nucleic acid" (PNA) refers to an antisense molecule or anti-gene agent which
30 comprises an oligonucleotide of at least about 5 nucleotides in length linked to a peptide backbone of amino acid residues ending in lysine. The terminal lysine confers solubility to the composition. PNAs preferentially bind complementary single stranded DNA or RNA and stop transcript elongation, and may be pegylated to extend their lifespan in the cell.

"Post-translational modification" of an HYENZ may involve lipidation, glycosylation,
35 phosphorylation, acetylation, racemization, proteolytic cleavage, and other modifications known in the

art. These processes may occur synthetically or biochemically. Biochemical modifications will vary by cell type depending on the enzymatic milieu of HYENZ.

"Probe" refers to nucleic acid sequences encoding HYENZ, their complements, or fragments thereof, which are used to detect identical, allelic or related nucleic acid sequences. Probes are isolated oligonucleotides or polynucleotides attached to a detectable label or reporter molecule. Typical labels include radioactive isotopes, ligands, chemiluminescent agents, and enzymes. "Primers" are short nucleic acids, usually DNA oligonucleotides, which may be annealed to a target polynucleotide by complementary base-pairing. The primer may then be extended along the target DNA strand by a DNA polymerase enzyme. Primer pairs can be used for amplification (and identification) of a nucleic acid sequence, e.g., by the polymerase chain reaction (PCR).

Probes and primers as used in the present invention typically comprise at least 15 contiguous nucleotides of a known sequence. In order to enhance specificity, longer probes and primers may also be employed, such as probes and primers that comprise at least 20, 25, 30, 40, 50, 60, 70, 80, 90, 100, or at least 150 consecutive nucleotides of the disclosed nucleic acid sequences. Probes and primers may be considerably longer than these examples, and it is understood that any length supported by the specification, including the tables, figures, and Sequence Listing, may be used.

Methods for preparing and using probes and primers are described in the references, for example Sambrook, J. et al. (1989) Molecular Cloning: A Laboratory Manual, 2nd ed., vol. 1-3, Cold Spring Harbor Press, Plainview NY; Ausubel, F.M. et al. (1987) Current Protocols in Molecular Biology, Greene Publ. Assoc. & Wiley-Intersciences, New York NY; Innis, M. et al. (1990) PCR Protocols, A Guide to Methods and Applications, Academic Press, San Diego CA. PCR primer pairs can be derived from a known sequence, for example, by using computer programs intended for that purpose such as Primer (Version 0.5, 1991, Whitehead Institute for Biomedical Research, Cambridge MA).

Oligonucleotides for use as primers are selected using software known in the art for such purpose. For example, OLIGO 4.06 software is useful for the selection of PCR primer pairs of up to 100 nucleotides each, and for the analysis of oligonucleotides and larger polynucleotides of up to 5,000 nucleotides from an input polynucleotide sequence of up to 32 kilobases. Similar primer selection programs have incorporated additional features for expanded capabilities. For example, the PrimOU primer selection program (available to the public from the Genome Center at University of Texas South West Medical Center, Dallas TX) is capable of choosing specific primers from megabase sequences and is thus useful for designing primers on a genome-wide scope. The Primer3 primer selection program (available to the public from the Whitehead Institute/MIT Center for Genome Research, Cambridge MA) allows the user to input a "mispriming library," in which sequences to avoid as primer

binding sites are user-specified. Primer3 is useful, in particular, for the selection of oligonucleotides for microarrays. (The source code for the latter two primer selection programs may also be obtained from their respective sources and modified to meet the user's specific needs.) The PrimeGen program (available to the public from the UK Human Genome Mapping Project Resource Centre, Cambridge UK) designs primers based on multiple sequence alignments, thereby allowing selection of primers that hybridize to either the most conserved or least conserved regions of aligned nucleic acid sequences. Hence, this program is useful for identification of both unique and conserved oligonucleotides and polynucleotide fragments. The oligonucleotides and polynucleotide fragments identified by any of the above selection methods are useful in hybridization technologies, for example, as PCR or sequencing primers, microarray elements, or specific probes to identify fully or partially complementary polynucleotides in a sample of nucleic acids. Methods of oligonucleotide selection are not limited to those described above.

A "recombinant nucleic acid" is a sequence that is not naturally occurring or has a sequence that is made by an artificial combination of two or more otherwise separated segments of sequence. This artificial combination is often accomplished by chemical synthesis or, more commonly, by the artificial manipulation of isolated segments of nucleic acids, e.g., by genetic engineering techniques such as those described in Sambrook, *supra*. The term recombinant includes nucleic acids that have been altered solely by addition, substitution, or deletion of a portion of the nucleic acid. Frequently, a recombinant nucleic acid may include a nucleic acid sequence operably linked to a promoter sequence. Such a recombinant nucleic acid may be part of a vector that is used, for example, to transform a cell.

Alternatively, such recombinant nucleic acids may be part of a viral vector, e.g., based on a vaccinia virus, that could be used to vaccinate a mammal wherein the recombinant nucleic acid is expressed, inducing a protective immunological response in the mammal.

A "regulatory element" refers to a nucleic acid sequence usually derived from untranslated regions of a gene and includes enhancers, promoters, introns, and 5' and 3' untranslated regions (UTRs). Regulatory elements interact with host or viral proteins which control transcription, translation, or RNA stability.

"Reporter molecules" are chemical or biochemical moieties used for labeling a nucleic acid, amino acid, or antibody. Reporter molecules include radionuclides; enzymes; fluorescent, chemiluminescent, or chromogenic agents; substrates; cofactors; inhibitors; magnetic particles; and other moieties known in the art.

An "RNA equivalent," in reference to a DNA sequence, is composed of the same linear sequence of nucleotides as the reference DNA sequence with the exception that all occurrences of the nitrogenous base thymine are replaced with uracil, and the sugar backbone is composed of ribose

instead of deoxyribose.

The term "sample" is used in its broadest sense. A sample suspected of containing nucleic acids encoding HYENZ, or fragments thereof, or HYENZ itself, may comprise a bodily fluid; an extract from a cell, chromosome, organelle, or membrane isolated from a cell; a cell; genomic DNA, RNA, or cDNA, in solution or bound to a substrate; a tissue; a tissue print; etc.

The terms "specific binding" and "specifically binding" refer to that interaction between a protein or peptide and an agonist, an antibody, an antagonist, a small molecule, or any natural or synthetic binding composition. The interaction is dependent upon the presence of a particular structure of the protein, e.g., the antigenic determinant or epitope, recognized by the binding molecule. For example, if an antibody is specific for epitope "A," the presence of a polypeptide comprising the epitope A, or the presence of free unlabeled A, in a reaction containing free labeled A and the antibody will reduce the amount of labeled A that binds to the antibody.

The term "substantially purified" refers to nucleic acid or amino acid sequences that are removed from their natural environment and are isolated or separated, and are at least 60% free, preferably at least 75% free, and most preferably at least 90% free from other components with which they are naturally associated.

A "substitution" refers to the replacement of one or more amino acid residues or nucleotides by different amino acid residues or nucleotides, respectively.

"Substrate" refers to any suitable rigid or semi-rigid support including membranes, filters, chips, slides, wafers, fibers, magnetic or nonmagnetic beads, gels, tubing, plates, polymers, microparticles and capillaries. The substrate can have a variety of surface forms, such as wells, trenches, pins, channels and pores, to which polynucleotides or polypeptides are bound.

A "transcript image" refers to the collective pattern of gene expression by a particular cell type or tissue under given conditions at a given time.

"Transformation" describes a process by which exogenous DNA is introduced into a recipient cell. Transformation may occur under natural or artificial conditions according to various methods well known in the art, and may rely on any known method for the insertion of foreign nucleic acid sequences into a prokaryotic or eukaryotic host cell. The method for transformation is selected based on the type of host cell being transformed and may include, but is not limited to, bacteriophage or viral infection, electroporation, heat shock, lipofection, and particle bombardment. The term "transformed" cells includes stably transformed cells in which the inserted DNA is capable of replication either as an autonomously replicating plasmid or as part of the host chromosome, as well as transiently transformed cells which express the inserted DNA or RNA for limited periods of time.

A "transgenic organism," as used herein, is any organism, including but not limited to

animals and plants, in which one or more of the cells of the organism contains heterologous nucleic acid introduced by way of human intervention, such as by transgenic techniques well known in the art. The nucleic acid is introduced into the cell, directly or indirectly by introduction into a precursor of the cell, by way of deliberate genetic manipulation, such as by microinjection or by infection with a recombinant virus. The term genetic manipulation does not include classical cross-breeding, or in vitro fertilization, but rather is directed to the introduction of a recombinant DNA molecule. The transgenic organisms contemplated in accordance with the present invention include bacteria, cyanobacteria, fungi, plants, and animals. The isolated DNA of the present invention can be introduced into the host by methods known in the art, for example infection, transfection, transformation or transconjugation. Techniques for transferring the DNA of the present invention into such organisms are widely known and provided in references such as Sambrook, J. et al. (1989), supra.

A "variant" of a particular nucleic acid sequence is defined as a nucleic acid sequence having at least 40% sequence identity to the particular nucleic acid sequence over a certain length of one of the nucleic acid sequences using blastn with the "BLAST 2 Sequences" tool Version 2.0.9 (May-07-1999) set at default parameters. Such a pair of nucleic acids may show, for example, at least 50%, at least 60%, at least 70%, at least 80%, at least 85%, at least 90%, at least 95% or at least 98% or greater sequence identity over a certain defined length. A variant may be described as, for example, an "allelic" (as defined above), "splice," "species," or "polymorphic" variant. A splice variant may have significant identity to a reference molecule, but will generally have a greater or lesser number of polynucleotides due to alternative splicing of exons during mRNA processing. The corresponding polypeptide may possess additional functional domains or lack domains that are present in the reference molecule. Species variants are polynucleotide sequences that vary from one species to another. The resulting polypeptides generally will have significant amino acid identity relative to each other. A polymorphic variant is a variation in the polynucleotide sequence of a particular gene between individuals of a given species. Polymorphic variants also may encompass "single nucleotide polymorphisms" (SNPs) in which the polynucleotide sequence varies by one nucleotide base. The presence of SNPs may be indicative of, for example, a certain population, a disease state, or a propensity for a disease state.

A "variant" of a particular polypeptide sequence is defined as a polypeptide sequence having at least 40% sequence identity to the particular polypeptide sequence over a certain length of one of the polypeptide sequences using blastp with the "BLAST 2 Sequences" tool Version 2.0.9 (May-07-1999) set at default parameters. Such a pair of polypeptides may show, for example, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, or at least 98% or greater sequence identity over a certain defined length of one of the polypeptides.

THE INVENTION

The invention is based on the discovery of new human hydrolytic enzymes (HYENZ), the polynucleotides encoding HYENZ, and the use of these compositions for the diagnosis, treatment, or prevention of neurological disorders, immune system disorders, genetic disorders, and cell proliferation disorders, including cancer.

5 Table 1 lists the Incyte clones used to assemble full length nucleotide sequences encoding HYENZ. Columns 1 and 2 show the sequence identification numbers (SEQ ID NOs) of the polypeptide and nucleotide sequences, respectively. Column 3 shows the clone IDs of the Incyte clones in which nucleic acids encoding each HYENZ were identified, and column 4 shows the cDNA libraries from which these clones were isolated. Column 5 shows Incyte clones and their corresponding cDNA
10 libraries. Clones for which cDNA libraries are not indicated were derived from pooled cDNA libraries. In some cases, GenBank sequence identifiers are also shown in column 5. The Incyte clones, and GenBank cDNA sequences, where indicated, in column 5 were used to assemble the consensus nucleotide sequence of each HYENZ and are useful as fragments in hybridization technologies.

The columns of Table 2 show various properties of each of the polypeptides of the invention:
15 column 1 references the SEQ ID NO; column 2 shows the number of amino acid residues in each polypeptide; column 3 shows potential phosphorylation sites; column 4 shows potential glycosylation sites; column 5 shows the amino acid residues comprising signature sequences and motifs; column 6 shows homologous sequences as identified by BLAST analysis; and column 7 shows analytical methods and in some cases, searchable databases to which the analytical methods were applied. The methods of
20 column 7 were used to characterize each polypeptide through sequence homology and protein motifs.

The columns of Table 3 show the tissue-specificity and diseases, disorders, or conditions associated with nucleotide sequences encoding HYENZ. The first column of Table 3 lists the nucleotide SEQ ID NOs. Column 2 lists fragments of the nucleotide sequences of column 1. These fragments are useful, for example, in hybridization or amplification technologies to identify SEQ ID
25 NO:15-28 and to distinguish between SEQ ID NO:15-28 and related polynucleotide sequences. The polypeptides encoded by these fragments are useful, for example, as immunogenic peptides. Column 3 lists tissue categories which express HYENZ as a fraction of total tissues expressing HYENZ. Column 4 lists diseases, disorders, or conditions associated with those tissues expressing HYENZ as a fraction of total tissues expressing HYENZ. Column 5 lists the vectors used to subclone each cDNA library.

30 The columns of Table 4 show descriptions of the tissues used to construct the cDNA libraries from which cDNA clones encoding HYENZ were isolated. Column 1 references the nucleotide SEQ ID NOs, column 2 shows the cDNA libraries from which these clones were isolated, and column 3 shows the tissue origins and other descriptive information relevant to the cDNA libraries in column 2.

SEQ ID NO:19 maps to chromosome 1 within the interval from 75.3 to 81.6 centiMorgans.

This interval also contains a gene associated with T cell acute lymphocytic leukemia 1. This interval also contains ESTs associated with various hydrolytic enzymes, such as carnitine palmitoyltransferase II and fatty acid amide hydrolase (FAAH).

The invention also encompasses HYENZ variants. A preferred HYENZ variant is one which has at least about 80%, or alternatively at least about 90%, or even at least about 95% amino acid sequence identity to the HYENZ amino acid sequence, and which contains at least one functional or structural characteristic of HYENZ.

The invention also encompasses polynucleotides which encode HYENZ. In a particular embodiment, the invention encompasses a polynucleotide sequence comprising a sequence selected from the group consisting of SEQ ID NO:15-28, which encodes HYENZ. The polynucleotide sequences of SEQ ID NO:15-28, as presented in the Sequence Listing, embrace the equivalent RNA sequences, wherein occurrences of the nitrogenous base thymine are replaced with uracil, and the sugar backbone is composed of ribose instead of deoxyribose.

The invention also encompasses a variant of a polynucleotide sequence encoding HYENZ. In particular, such a variant polynucleotide sequence will have at least about 70%, or alternatively at least about 85%, or even at least about 95% polynucleotide sequence identity to the polynucleotide sequence encoding HYENZ. A particular aspect of the invention encompasses a variant of a polynucleotide sequence comprising a sequence selected from the group consisting of SEQ ID NO:15-28 which has at least about 70%, or alternatively at least about 85%, or even at least about 95% polynucleotide sequence identity to a nucleic acid sequence selected from the group consisting of SEQ ID NO:15-28. Any one of the polynucleotide variants described above can encode an amino acid sequence which contains at least one functional or structural characteristic of HYENZ.

It will be appreciated by those skilled in the art that as a result of the degeneracy of the genetic code, a multitude of polynucleotide sequences encoding HYENZ, some bearing minimal similarity to the polynucleotide sequences of any known and naturally occurring gene, may be produced. Thus, the invention contemplates each and every possible variation of polynucleotide sequence that could be made by selecting combinations based on possible codon choices. These combinations are made in accordance with the standard triplet genetic code as applied to the polynucleotide sequence of naturally occurring HYENZ, and all such variations are to be considered as being specifically disclosed.

Although nucleotide sequences which encode HYENZ and its variants are generally capable of hybridizing to the nucleotide sequence of the naturally occurring HYENZ under appropriately selected conditions of stringency, it may be advantageous to produce nucleotide sequences encoding HYENZ or its derivatives possessing a substantially different codon usage, e.g., inclusion of non-naturally occurring codons. Codons may be selected to increase the rate at which expression of the peptide

occurs in a particular prokaryotic or eukaryotic host in accordance with the frequency with which particular codons are utilized by the host. Other reasons for substantially altering the nucleotide sequence encoding HYENZ and its derivatives without altering the encoded amino acid sequences include the production of RNA transcripts having more desirable properties, such as a greater half-life, than transcripts produced from the naturally occurring sequence.

The invention also encompasses production of DNA sequences which encode HYENZ and HYENZ derivatives, or fragments thereof, entirely by synthetic chemistry. After production, the synthetic sequence may be inserted into any of the many available expression vectors and cell systems using reagents well known in the art. Moreover, synthetic chemistry may be used to introduce mutations into a sequence encoding HYENZ or any fragment thereof.

Also encompassed by the invention are polynucleotide sequences that are capable of hybridizing to the claimed polynucleotide sequences, and, in particular, to those shown in SEQ ID NO:15-28 and fragments thereof under various conditions of stringency. (See, e.g., Wahl, G.M. and S.L. Berger (1987) *Methods Enzymol.* 152:399-407; Kimmel, A.R. (1987) *Methods Enzymol.* 152:507-511.) Hybridization conditions, including annealing and wash conditions, are described in "Definitions."

Methods for DNA sequencing are well known in the art and may be used to practice any of the embodiments of the invention. The methods may employ such enzymes as the Klenow fragment of DNA polymerase I, SEQUENASE (US Biochemical, Cleveland OH), Taq polymerase (PE Biosystems, Foster City CA), thermostable T7 polymerase (Amersham Pharmacia Biotech, Piscataway NJ), or combinations of polymerases and proofreading exonucleases such as those found in the ELONGASE amplification system (Life Technologies, Gaithersburg MD). Preferably, sequence preparation is automated with machines such as the MICROLAB 2200 liquid transfer system (Hamilton, Reno NV), PTC200 thermal cycler (MJ Research, Watertown MA) and ABI CATALYST 800 thermal cycler (PE Biosystems). Sequencing is then carried out using either the ABI 373 or 377 DNA sequencing system (PE Biosystems), the MEGABACE 1000 DNA sequencing system (Molecular Dynamics, Sunnyvale CA), or other systems known in the art. The resulting sequences are analyzed using a variety of algorithms which are well known in the art. (See, e.g., Ausubel, F.M. (1997) Short Protocols in Molecular Biology, John Wiley & Sons, New York NY, unit 7.7; Meyers, R.A. (1995) Molecular Biology and Biotechnology, Wiley VCH, New York NY, pp. 856-853.)

The nucleic acid sequences encoding HYENZ may be extended utilizing a partial nucleotide sequence and employing various PCR-based methods known in the art to detect upstream sequences, such as promoters and regulatory elements. For example, one method which may be employed, restriction-site PCR, uses universal and nested primers to amplify unknown sequence from genomic

DNA within a cloning vector. (See, e.g., Sarkar, G. (1993) PCR Methods Applic. 2:318-322.)

Another method, inverse PCR, uses primers that extend in divergent directions to amplify unknown sequence from a circularized template. The template is derived from restriction fragments comprising a

known genomic locus and surrounding sequences. (See, e.g., Triglia, T. et al. (1988) Nucleic Acids

Res. 16:8186.) A third method, capture PCR, involves PCR amplification of DNA fragments adjacent

to known sequences in human and yeast artificial chromosome DNA. (See, e.g., Lagerstrom, M. et al.

(1991) PCR Methods Applic. 1:111-119.) In this method, multiple restriction enzyme digestions and

ligations may be used to insert an engineered double-stranded sequence into a region of unknown

sequence before performing PCR. Other methods which may be used to retrieve unknown sequences

are known in the art. (See, e.g., Parker, J.D. et al. (1991) Nucleic Acids Res. 19:3055-3060).

Additionally, one may use PCR, nested primers, and PROMOTERFINDER libraries (Clontech, Palo

Alto CA) to walk genomic DNA. This procedure avoids the need to screen libraries and is useful in

finding intron/exon junctions. For all PCR-based methods, primers may be designed using

commercially available software, such as OLIGO 4.06 Primer Analysis software (National Biosciences,

Plymouth MN) or another appropriate program, to be about 22 to 30 nucleotides in length, to have a

GC content of about 50% or more, and to anneal to the template at temperatures of about 68°C to

72°C.

When screening for full-length cDNAs, it is preferable to use libraries that have been

size-selected to include larger cDNAs. In addition, random-primed libraries, which often include

sequences containing the 5' regions of genes, are preferable for situations in which an oligo d(T) library

does not yield a full-length cDNA. Genomic libraries may be useful for extension of sequence into 5'

non-transcribed regulatory regions.

Capillary electrophoresis systems which are commercially available may be used to analyze the size or confirm the nucleotide sequence of sequencing or PCR products. In particular, capillary

sequencing may employ flowable polymers for electrophoretic separation, four different nucleotide-

specific, laser-stimulated fluorescent dyes, and a charge coupled device camera for detection of the

emitted wavelengths. Output/light intensity may be converted to electrical signal using appropriate

software (e.g., GENOTYPER and SEQUENCE NAVIGATOR, PE Biosystems), and the entire process

from loading of samples to computer analysis and electronic data display may be computer controlled.

Capillary electrophoresis is especially preferable for sequencing small DNA fragments which may be

present in limited amounts in a particular sample.

In another embodiment of the invention, polynucleotide sequences or fragments thereof which encode HYENZ may be cloned in recombinant DNA molecules that direct expression of HYENZ, or

fragments or functional equivalents thereof, in appropriate host cells. Due to the inherent degeneracy of

the genetic code, other DNA sequences which encode substantially the same or a functionally equivalent amino acid sequence may be produced and used to express HYENZ.

The nucleotide sequences of the present invention can be engineered using methods generally known in the art in order to alter HYENZ-encoding sequences for a variety of purposes including, but not limited to, modification of the cloning, processing, and/or expression of the gene product. DNA shuffling by random fragmentation and PCR reassembly of gene fragments and synthetic oligonucleotides may be used to engineer the nucleotide sequences. For example, oligonucleotide-mediated site-directed mutagenesis may be used to introduce mutations that create new restriction sites, alter glycosylation patterns, change codon preference, produce splice variants, and so forth.

The nucleotides of the present invention may be subjected to DNA shuffling techniques such as MOLECULARBREEDING (Maxygen Inc., Santa Clara CA; described in U.S. Patent Number 5,837,458; Chang, C.-C. et al. (1999) Nat. Biotechnol. 17:793-797; Christians, F.C. et al. (1999) Nat. Biotechnol. 17:259-264; and Cramer, A. et al. (1996) Nat. Biotechnol. 14:315-319) to alter or improve the biological properties of HYENZ, such as its biological or enzymatic activity or its ability to bind to other molecules or compounds. DNA shuffling is a process by which a library of gene variants is produced using PCR-mediated recombination of gene fragments. The library is then subjected to selection or screening procedures that identify those gene variants with the desired properties. These preferred variants may then be pooled and further subjected to recursive rounds of DNA shuffling and selection/screening. Thus, genetic diversity is created through "artificial" breeding and rapid molecular evolution. For example, fragments of a single gene containing random point mutations may be recombined, screened, and then reshuffled until the desired properties are optimized. Alternatively, fragments of a given gene may be recombined with fragments of homologous genes in the same gene family, either from the same or different species, thereby maximizing the genetic diversity of multiple naturally occurring genes in a directed and controllable manner.

In another embodiment, sequences encoding HYENZ may be synthesized, in whole or in part, using chemical methods well known in the art. (See, e.g., Caruthers, M.H. et al. (1980) Nucleic Acids Symp. Ser. 7:215-223; Horn, T. et al. (1980) Nucleic Acids Symp. Ser. 7:225-232.) Alternatively, HYENZ itself or a fragment thereof may be synthesized using chemical methods. For example, peptide synthesis can be performed using various solution-phase or solid-phase techniques. (See, e.g., Creighton, T. (1984) Proteins, Structures and Molecular Properties, WH Freeman, New York NY, pp. 55-60; and Roberge, J.Y. et al. (1995) Science 269:202-204.) Automated synthesis may be achieved using the ABI 431A peptide synthesizer (PE Biosystems). Additionally, the amino acid sequence of HYENZ, or any part thereof, may be altered during direct synthesis and/or combined with sequences from other proteins, or any part thereof, to produce a variant polypeptide or a polypeptide having a

sequence of a naturally occurring polypeptide.

The peptide may be substantially purified by preparative high performance liquid chromatography. (See, e.g., Chiez, R.M. and F.Z. Regnier (1990) *Methods Enzymol.* 182:392-421.) The composition of the synthetic peptides may be confirmed by amino acid analysis or by sequencing.
5 (See, e.g., Creighton, supra, pp. 28-53.)

In order to express a biologically active HYENZ, the nucleotide sequences encoding HYENZ or derivatives thereof may be inserted into an appropriate expression vector, i.e., a vector which contains the necessary elements for transcriptional and translational control of the inserted coding sequence in a suitable host. These elements include regulatory sequences, such as enhancers,
10 constitutive and inducible promoters, and 5' and 3' untranslated regions in the vector and in polynucleotide sequences encoding HYENZ. Such elements may vary in their strength and specificity. Specific initiation signals may also be used to achieve more efficient translation of sequences encoding HYENZ. Such signals include the ATG initiation codon and adjacent sequences, e.g. the Kozak sequence. In cases where sequences encoding HYENZ and its initiation codon and upstream regulatory
15 sequences are inserted into the appropriate expression vector, no additional transcriptional or translational control signals may be needed. However, in cases where only coding sequence, or a fragment thereof, is inserted, exogenous translational control signals including an in-frame ATG initiation codon should be provided by the vector. Exogenous translational elements and initiation codons may be of various origins, both natural and synthetic. The efficiency of expression may be
20 enhanced by the inclusion of enhancers appropriate for the particular host cell system used. (See, e.g., Scharf, D. et al. (1994) *Results Probl. Cell Differ.* 20:125-162.)

Methods which are well known to those skilled in the art may be used to construct expression vectors containing sequences encoding HYENZ and appropriate transcriptional and translational control elements. These methods include in vitro recombinant DNA techniques, synthetic techniques,
25 and in vivo genetic recombination. (See, e.g., Sambrook, J. et al. (1989) Molecular Cloning, A Laboratory Manual, Cold Spring Harbor Press, Plainview NY, ch. 4, 8, and 16-17; Ausubel, F.M. et al. (1995) Current Protocols in Molecular Biology, John Wiley & Sons, New York NY, ch. 9, 13, and 16.)

A variety of expression vector/host systems may be utilized to contain and express sequences
30 encoding HYENZ. These include, but are not limited to, microorganisms such as bacteria transformed with recombinant bacteriophage, plasmid, or cosmid DNA expression vectors; yeast transformed with yeast expression vectors; insect cell systems infected with viral expression vectors (e.g., baculovirus); plant cell systems transformed with viral expression vectors (e.g., cauliflower mosaic virus, CaMV, or tobacco mosaic virus, TMV) or with bacterial expression vectors (e.g., Ti or pBR322 plasmids); or

- animal cell systems. (See, e.g., Sambrook, supra; Ausubel, supra; Van Heeke, G. and S.M. Schuster (1989) J. Biol. Chem. 264:5503-5509; Bitter, G.A. et al. (1987) Methods Enzymol. 153:516-544; Scorer, C.A. et al. (1994) Bio/Technology 12:181-184; Engelhard, E.K. et al. (1994) Proc. Natl. Acad. Sci. USA 91:3224-3227; Sandig, V. et al. (1996) Hum. Gene Ther. 7:1937-1945; Takamatsu, N. (1987) EMBO J. 6:307-311; Coruzzi, G. et al. (1984) EMBO J. 3:1671-1680; Broglie, R. et al. (1984) Science 224:838-843; Winter, J. et al. (1991) Results Probl. Cell Differ. 17:85-105; The McGraw Hill Yearbook of Science and Technology (1992) McGraw Hill, New York NY, pp. 191-196; Logan, J. and T. Shenk (1984) Proc. Natl. Acad. Sci. USA 81:3655-3659; and Harrington, J.J. et al. (1997) Nat. Genet. 15:345-355.) Expression vectors derived from retroviruses, adenoviruses, or herpes or vaccinia viruses, or from various bacterial plasmids, may be used for delivery of nucleotide sequences to the targeted organ, tissue, or cell population. (See, e.g., Di Nicola, M. et al. (1998) Cancer Gen. Ther. 5(6):350-356; Yu, M. et al. (1993) Proc. Natl. Acad. Sci. USA 90(13):6340-6344; Buller, R.M. et al. (1985) Nature 317(6040):813-815; McGregor, D.P. et al. (1994) Mol. Immunol. 31(3):219-226; and Verma, I.M. and N. Somia (1997) Nature 389:239-242.)
- The invention is not limited by the host cell employed.

- In bacterial systems, a number of cloning and expression vectors may be selected depending upon the use intended for polynucleotide sequences encoding HYENZ. For example, routine cloning, subcloning, and propagation of polynucleotide sequences encoding HYENZ can be achieved using a multifunctional E. coli vector such as PBLUESCRIPT (Stratagene, La Jolla CA) or PSPORT1 plasmid (Life Technologies). Ligation of sequences encoding HYENZ into the vector's multiple cloning site disrupts the *lacZ* gene, allowing a colorimetric screening procedure for identification of transformed bacteria containing recombinant molecules. In addition, these vectors may be useful for in vitro transcription, dideoxy sequencing, single strand rescue with helper phage, and creation of nested deletions in the cloned sequence. (See, e.g., Van Heeke, G. and S.M. Schuster (1989) J. Biol. Chem. 264:5503-5509.) When large quantities of HYENZ are needed, e.g. for the production of antibodies, vectors which direct high level expression of HYENZ may be used. For example, vectors containing the strong, inducible T5 or T7 bacteriophage promoter may be used.

- Yeast expression systems may be used for production of HYENZ. A number of vectors containing constitutive or inducible promoters, such as alpha factor, alcohol oxidase, and PGH promoters, may be used in the yeast Saccharomyces cerevisiae or Pichia pastoris. In addition, such vectors direct either the secretion or intracellular retention of expressed proteins and enable integration of foreign sequences into the host genome for stable propagation. (See, e.g., Ausubel, 1995, supra; Bitter, supra; and Scorer, supra.)

- Plant systems may also be used for expression of HYENZ. Transcription of sequences encoding HYENZ may be driven viral promoters, e.g., the 35S and 19S promoters of CaMV used alone

or in combination with the omega leader sequence from TMV (Takamatsu, N. (1987) EMBO J. 6:307-311). Alternatively, plant promoters such as the small subunit of RUBISCO or heat shock promoters may be used. (See, e.g., Coruzzi, supra; Broglie, supra; and Winter, supra.) These constructs can be introduced into plant cells by direct DNA transformation or pathogen-mediated transfection. (See, e.g., The McGraw Hill Yearbook of Science and Technology (1992) McGraw Hill, New York NY, pp. 191-196.)

In mammalian cells, a number of viral-based expression systems may be utilized. In cases where an adenovirus is used as an expression vector, sequences encoding HYENZ may be ligated into an adenovirus transcription/translation complex consisting of the late promoter and tripartite leader sequence. Insertion in a non-essential E1 or E3 region of the viral genome may be used to obtain infective virus which expresses HYENZ in host cells. (See, e.g., Logan, J. and T. Shenk (1984) Proc. Natl. Acad. Sci. USA 81:3655-3659.) In addition, transcription enhancers, such as the Rous sarcoma virus (RSV) enhancer, may be used to increase expression in mammalian host cells. SV40 or EBV-based vectors may also be used for high-level protein expression.

Human artificial chromosomes (HACs) may also be employed to deliver larger fragments of DNA than can be contained in and expressed from a plasmid. HACs of about 6 kb to 10 Mb are constructed and delivered via conventional delivery methods (liposomes, polycationic amino polymers, or vesicles) for therapeutic purposes. (See, e.g., Harrington, J.J. et al. (1997) Nat. Genet. 15:345-355.)

For long term production of recombinant proteins in mammalian systems, stable expression of HYENZ in cell lines is preferred. For example, sequences encoding HYENZ can be transformed into cell lines using expression vectors which may contain viral origins of replication and/or endogenous expression elements and a selectable marker gene on the same or on a separate vector. Following the introduction of the vector, cells may be allowed to grow for about 1 to 2 days in enriched media before being switched to selective media. The purpose of the selectable marker is to confer resistance to a selective agent, and its presence allows growth and recovery of cells which successfully express the introduced sequences. Resistant clones of stably transformed cells may be propagated using tissue culture techniques appropriate to the cell type.

Any number of selection systems may be used to recover transformed cell lines. These include, but are not limited to, the herpes simplex virus thymidine kinase and adenine phosphoribosyltransferase genes, for use in *tk⁻* and *ap^r* cells, respectively. (See, e.g., Wigler, M. et al. (1977) Cell 11:223-232; Lowy, I. et al. (1980) Cell 22:817-823.) Also, antimetabolite, antibiotic, or herbicide resistance can be used as the basis for selection. For example, *dhfr* confers resistance to methotrexate; *neo* confers resistance to the aminoglycosides neomycin and G-418; and *als* and *pat* confer resistance to chlorsulfuron and phosphinotricin acetyltransferase, respectively. (See, e.g., Wigler, M. et al. (1980)

Proc. Natl. Acad. Sci. USA 77:3567-3570; Colbere-Garapin, F. et al. (1981) J. Mol. Biol. 150:1-14.) Additional selectable genes have been described, e.g., *trpB* and *hisD*, which alter cellular requirements for metabolites. (See, e.g., Hartman, S.C. and R.C. Mulligan (1988) Proc. Natl. Acad. Sci. USA 85:8047-8051.) Visible markers, e.g., anthocyanins, green fluorescent proteins (GFP; Clontech), β glucuronidase and its substrate β -glucuronide, or luciferase and its substrate luciferin may be used. These markers can be used not only to identify transformants, but also to quantify the amount of transient or stable protein expression attributable to a specific vector system. (See, e.g., Rhodes, C.A. (1995) Methods Mol. Biol. 55:121-131.)

Although the presence/absence of marker gene expression suggests that the gene of interest is also present, the presence and expression of the gene may need to be confirmed. For example, if the sequence encoding HYENZ is inserted within a marker gene sequence, transformed cells containing sequences encoding HYENZ can be identified by the absence of marker gene function. Alternatively, a marker gene can be placed in tandem with a sequence encoding HYENZ under the control of a single promoter. Expression of the marker gene in response to induction or selection usually indicates expression of the tandem gene as well.

In general, host cells that contain the nucleic acid sequence encoding HYENZ and that express HYENZ may be identified by a variety of procedures known to those of skill in the art. These procedures include, but are not limited to, DNA-DNA or DNA-RNA hybridizations, PCR amplification, and protein bioassay or immunoassay techniques which include membrane, solution, or chip based technologies for the detection and/or quantification of nucleic acid or protein sequences.

Immunological methods for detecting and measuring the expression of HYENZ using either specific polyclonal or monoclonal antibodies are known in the art. Examples of such techniques include enzyme-linked immunosorbent assays (ELISAs), radioimmunoassays (RIAs), and fluorescence activated cell sorting (FACS). A two-site, monoclonal-based immunoassay utilizing monoclonal antibodies reactive to two non-interfering epitopes on HYENZ is preferred, but a competitive binding assay may be employed. These and other assays are well known in the art. (See, e.g., Hampton, R. et al. (1990) Serological Methods, a Laboratory Manual, APS Press, St. Paul MN, Sect. IV; Coligan, J.E. et al. (1997) Current Protocols in Immunology, Greene Pub. Associates and Wiley-Interscience, New York NY; and Pound, J.D. (1998) Immunochemical Protocols, Humana Press, Totowa NJ.)

A wide variety of labels and conjugation techniques are known by those skilled in the art and may be used in various nucleic acid and amino acid assays. Means for producing labeled hybridization or PCR probes for detecting sequences related to polynucleotides encoding HYENZ include oligolabeling, nick translation, end-labeling, or PCR amplification using a labeled nucleotide. Alternatively, the sequences encoding HYENZ, or any fragments thereof, may be cloned into a vector

for the production of an mRNA probe. Such vectors are known in the art, are commercially available, and may be used to synthesize RNA probes in vitro by addition of an appropriate RNA polymerase such as T7, T3, or SP6 and labeled nucleotides. These procedures may be conducted using a variety of commercially available kits, such as those provided by Amersham Pharmacia Biotech, Promega (Madison WI), and US Biochemical. Suitable reporter molecules or labels which may be used for ease of detection include radionuclides, enzymes, fluorescent, chemiluminescent, or chromogenic agents, as well as substrates, cofactors, inhibitors, magnetic particles, and the like.

Host cells transformed with nucleotide sequences encoding HYENZ may be cultured under conditions suitable for the expression and recovery of the protein from cell culture. The protein produced by a transformed cell may be secreted or retained intracellularly depending on the sequence and/or the vector used. As will be understood by those of skill in the art, expression vectors containing polynucleotides which encode HYENZ may be designed to contain signal sequences which direct secretion of HYENZ through a prokaryotic or eukaryotic cell membrane.

In addition, a host cell strain may be chosen for its ability to modulate expression of the inserted sequences or to process the expressed protein in the desired fashion. Such modifications of the polypeptide include, but are not limited to, acetylation, carboxylation, glycosylation, phosphorylation, lipidation, and acylation. Post-translational processing which cleaves a "prepro" or "pro" form of the protein may also be used to specify protein targeting, folding, and/or activity. Different host cells which have specific cellular machinery and characteristic mechanisms for post-translational activities (e.g., CHO, HeLa, MDCK, HEK293, and WI38) are available from the American Type Culture Collection (ATCC, Manassas VA) and may be chosen to ensure the correct modification and processing of the foreign protein.

In another embodiment of the invention, natural, modified, or recombinant nucleic acid sequences encoding HYENZ may be ligated to a heterologous sequence resulting in translation of a fusion protein in any of the aforementioned host systems. For example, a chimeric HYENZ protein containing a heterologous moiety that can be recognized by a commercially available antibody may facilitate the screening of peptide libraries for inhibitors of HYENZ activity. Heterologous protein and peptide moieties may also facilitate purification of fusion proteins using commercially available affinity matrices. Such moieties include, but are not limited to, glutathione S-transferase (GST), maltose binding protein (MBP), thioredoxin (Trx), calmodulin binding peptide (CBP), 6-His, FLAG, *c-myc*, and hemagglutinin (HA). GST, MBP, Trx, CBP, and 6-His enable purification of their cognate fusion proteins on immobilized glutathione, maltose, phenylarsine oxide, calmodulin, and metal-chelate resins, respectively. FLAG, *c-myc*, and hemagglutinin (HA) enable immunoaffinity purification of fusion proteins using commercially available monoclonal and polyclonal antibodies that specifically recognize

these epitope tags. A fusion protein may also be engineered to contain a proteolytic cleavage site located between the HYENZ encoding sequence and the heterologous protein sequence, so that HYENZ may be cleaved away from the heterologous moiety following purification. Methods for fusion protein expression and purification are discussed in Ausubel (1995, supra, ch. 10). A variety of commercially available kits may also be used to facilitate expression and purification of fusion proteins.

In a further embodiment of the invention, synthesis of radiolabeled HYENZ may be achieved in vitro using the TNT rabbit reticulocyte lysate or wheat germ extract system (Promega). These systems couple transcription and translation of protein-coding sequences operably associated with the T7, T3, or SP6 promoters. Translation takes place in the presence of a radiolabeled amino acid precursor, for example, ³⁵S-methionine.

HYENZ of the present invention or fragments thereof may be used to screen for compounds that specifically bind to HYENZ. At least one and up to a plurality of test compounds may be screened for specific binding to HYENZ. Examples of test compounds include antibodies, oligonucleotides, proteins (e.g., receptors), or small molecules.

In one embodiment, the compound thus identified is closely related to the natural ligand of HYENZ, e.g., a ligand or fragment thereof, a natural substrate, a structural or functional mimetic, or a natural binding partner. (See, e.g., Coligan, J.E. et al. (1991) Current Protocols in Immunology 1(2): Chapter 5.) Similarly, the compound can be closely related to the natural receptor to which HYENZ binds, or to at least a fragment of the receptor, e.g., the ligand binding site. In either case, the compound can be rationally designed using known techniques. In one embodiment, screening for these compounds involves producing appropriate cells which express HYENZ, either as a secreted protein or on the cell membrane. Preferred cells include cells from mammals, yeast, Drosophila, or E. coli. Cells expressing HYENZ or cell membrane fractions which contain HYENZ are then contacted with a test compound and binding, stimulation, or inhibition of activity of either HYENZ or the compound is analyzed.

An assay may simply test binding of a test compound to the polypeptide, wherein binding is detected by a fluorophore, radioisotope, enzyme conjugate, or other detectable label. For example, the assay may comprise the steps of combining at least one test compound with HYENZ, either in solution or affixed to a solid support, and detecting the binding of HYENZ to the compound.

Alternatively, the assay may detect or measure binding of a test compound in the presence of a labeled competitor. Additionally, the assay may be carried out using cell-free preparations, chemical libraries, or natural product mixtures, and the test compound(s) may be free in solution or affixed to a solid support.

HYENZ of the present invention or fragments thereof may be used to screen for compounds that modulate the activity of HYENZ. Such compounds may include agonists, antagonists, or partial

or inverse agonists. In one embodiment, an assay is performed under conditions permissive for HYENZ activity, wherein HYENZ is combined with at least one test compound, and the activity of HYENZ in the presence of a test compound is compared with the activity of HYENZ in the absence of the test compound. A change in the activity of HYENZ in the presence of the test compound is indicative of a compound that modulates the activity of HYENZ. Alternatively, a test compound is combined with an in vitro or cell-free system comprising HYENZ under conditions suitable for HYENZ activity, and the assay is performed. In either of these assays, a test compound which modulates the activity of HYENZ may do so indirectly and need not come in direct contact with the test compound. At least one and up to a plurality of test compounds may be screened.

In another embodiment, polynucleotides encoding HYENZ or their mammalian homologs may be "knocked out" in an animal model system using homologous recombination in embryonic stem (ES) cells. Such techniques are well known in the art and are useful for the generation of animal models of human disease. (See, e.g., U.S. Patent No. 5,175,383 and U.S. Patent No. 5,767,337.) For example, mouse ES cells, such as the mouse 129/SvJ cell line, are derived from the early mouse embryo and grown in culture. The ES cells are transformed with a vector containing the gene of interest disrupted by a marker gene, e.g., the neomycin phosphotransferase gene (neo; Capecchi, M.R. (1989) Science 244:1288-1292). The vector integrates into the corresponding region of the host genome by homologous recombination. Alternatively, homologous recombination takes place using the Cre-loxP system to knockout a gene of interest in a tissue- or developmental stage-specific manner (Marth, J.D. (1996) Clin. Invest. 97:1999-2002; Wagner, K.U. et al. (1997) Nucleic Acids Res. 25:4323-4330). Transformed ES cells are identified and microinjected into mouse cell blastocysts such as those from the C57BL/6 mouse strain. The blastocysts are surgically transferred to pseudopregnant dams, and the resulting chimeric progeny are genotyped and bred to produce heterozygous or homozygous strains. Transgenic animals thus generated may be tested with potential therapeutic or toxic agents.

Polynucleotides encoding HYENZ may also be manipulated in vitro in ES cells derived from human blastocysts. Human ES cells have the potential to differentiate into at least eight separate cell lineages including endoderm, mesoderm, and ectodermal cell types. These cell lineages differentiate into, for example, neural cells, hematopoietic lineages, and cardiomyocytes (Thomson, J.A. et al. (1998) Science 282:1145-1147).

Polynucleotides encoding HYENZ can also be used to create "knockin" humanized animals (pigs) or transgenic animals (mice or rats) to model human disease. With knockin technology, a region of a polynucleotide encoding HYENZ is injected into animal ES cells, and the injected sequence integrates into the animal cell genome. Transformed cells are injected into blastulae, and the blastulae are implanted as described above. Transgenic progeny or inbred lines are studied and

treated with potential pharmaceutical agents to obtain information on treatment of a human disease. Alternatively, a mammal inbred to overexpress HYENZ, e.g., by secreting HYENZ in its milk, may also serve as a convenient source of that protein (Janne, J. et al. (1998) *Biotechnol. Annu. Rev.* 4:55-74).

5 THERAPEUTICS

Chemical and structural similarity, e.g., in the context of sequences and motifs, exists between regions of HYENZ and hydrolytic enzymes. In addition, the expression of HYENZ is closely associated with cancerous, cardiovascular, dermatologic, developmental, endocrine, gastrointestinal, hematopoietic/immune, inflamed, nervous, proliferating, reproductive, and urologic
10 tissues. Therefore, HYENZ appears to play a role in neurological disorders, immune system disorders, genetic disorders, and cell proliferation disorders, including cancer. In the treatment of disorders associated with increased HYENZ expression or activity, it is desirable to decrease the expression or activity of HYENZ. In the treatment of disorders associated with decreased HYENZ expression or activity, it is desirable to increase the expression or activity of HYENZ.

15 Therefore, in one embodiment, HYENZ or a fragment or derivative thereof may be administered to a subject to treat or prevent a disorder associated with decreased expression or activity of HYENZ. Examples of such disorders include, but are not limited to, a neurological disorder, such as epilepsy, ischemic cerebrovascular disease, stroke, cerebral neoplasms, Alzheimer's disease, Pick's disease, Huntington's disease, dementia, Parkinson's disease and other extrapyramidal
20 disorders, amyotrophic lateral sclerosis and other motor neuron disorders, progressive neural muscular atrophy, retinitis pigmentosa, hereditary ataxias, multiple sclerosis and other demyelinating diseases, bacterial and viral meningitis, brain abscess, subdural empyema, epidural abscess, suppurative intracranial thrombophlebitis, myelitis and radiculitis, viral central nervous system disease; prion diseases including kuru, Creutzfeldt-Jakob disease, and Gerstmann-Straussler-Scheinker syndrome;
25 fatal familial insomnia, nutritional and metabolic diseases of the nervous system, neurofibromatosis, tuberous sclerosis, cerebelloretinal hemangioblastomatosis, encephalotrigeminal syndrome, mental retardation and other developmental disorder of the central nervous system, cerebral palsy, a neuroskeletal disorder, an autonomic nervous system disorder, a cranial nerve disorder, a spinal cord disease, muscular dystrophy and other neuromuscular disorder, a peripheral nervous system disorder,
30 dermatomyositis and polymyositis; inherited, metabolic, endocrine, and toxic myopathy; myasthenia gravis, periodic paralysis; a mental disorder including mood, anxiety, and schizophrenic disorders; seasonal affective disorder (SAD); akathisia, amnesia, catatonia, diabetic neuropathy, tardive dyskinesia, dystonias, paranoid psychoses, postherpetic neuralgia, and Tourette's disorder; an immune system disorder, such as inflammation, actinic keratosis, acquired immunodeficiency
35 syndrome (AIDS), Addison's disease, adult respiratory distress syndrome, allergies, ankylosing

spondylitis, amyloidosis, anemia, arteriosclerosis, asthma, atherosclerosis, autoimmune hemolytic anemia, autoimmune thyroiditis, bronchitis, bursitis, cholecystitis, cirrhosis, contact dermatitis, Crohn's disease, atopic dermatitis, dermatomyositis, diabetes mellitus, emphysema, erythroblastosis fetalis, erythema nodosum, atrophic gastritis, glomerulonephritis, Goodpasture's syndrome, gout, Graves' disease, Hashimoto's thyroiditis, paroxysmal nocturnal hemoglobinuria, hepatitis, hypereosinophilia, irritable bowel syndrome, episodic lymphopenia with lymphocytotoxins, mixed connective tissue disease (MCTD), multiple sclerosis, myasthenia gravis, myocardial or pericardial inflammation, myelofibrosis, osteoarthritis, osteoporosis, pancreatitis, polycythemia vera, polymyositis, psoriasis, Reiter's syndrome, rheumatoid arthritis, scleroderma, Sjögren's syndrome, systemic anaphylaxis, systemic lupus erythematosus, systemic sclerosis, primary thrombocythemia, thrombocytopenic purpura, ulcerative colitis, uveitis, Werner syndrome, complications of cancer, hemodialysis, and extracorporeal circulation, trauma, and hematopoietic cancer including lymphoma, leukemia, and myeloma; a genetic disorder, such as GM1-gangliosidosis, Niemann-Pick disease, adrenoleukodystrophy, Alport's syndrome, choroideremia, Duchenne and Becker muscular dystrophy, Down's syndrome, cystic fibrosis, chronic granulomatous disease, Gaucher's disease, Huntington's chorea, Marfan's syndrome, muscular dystrophy, myotonic dystrophy, pycnodysostosis, Refsum's syndrome, retinoblastoma, sickle cell anemia, thalassemia, Werner syndrome, von Willebrand's disease, von Hippel-Lindau syndrome, Wilms' tumor, Zellweger syndrome, peroxisomal acyl-CoA oxidase deficiency, peroxisomal thiolase deficiency, peroxisomal bifunctional protein deficiency, mitochondrial carnitine palmitoyl transferase and carnitine deficiency, mitochondrial very-long-chain acyl-CoA dehydrogenase deficiency, mitochondrial medium-chain acyl-CoA dehydrogenase deficiency, mitochondrial short-chain acyl-CoA dehydrogenase deficiency, mitochondrial electron transport flavoprotein and electron transport flavoprotein:ubiquinone oxidoreductase deficiency, mitochondrial trifunctional protein deficiency, and mitochondrial short-chain 3-hydroxyacyl-CoA dehydrogenase deficiency; and a cell proliferation disorder, such as actinic keratosis, arteriosclerosis, atherosclerosis, bursitis, cirrhosis, hepatitis, mixed connective tissue disease (MCTD), myelofibrosis, paroxysmal nocturnal hemoglobinuria, polycythemia vera, psoriasis, primary thrombocythemia, and cancers including adenocarcinoma, leukemia, lymphoma, melanoma, myeloma, sarcoma, teratocarcinoma, and, in particular, a cancer of the adrenal gland, bladder, bone, bone marrow, brain, breast, cervix, gall bladder, ganglia, gastrointestinal tract, heart, kidney, liver, lung, muscle, ovary, pancreas, parathyroid, penis, prostate, salivary glands, skin, spleen, testis, thymus, thyroid, and uterus.

In another embodiment, a vector capable of expressing HYENZ or a fragment or derivative thereof may be administered to a subject to treat or prevent a disorder associated with decreased expression or activity of HYENZ including, but not limited to, those described above.

In a further embodiment, a composition comprising a substantially purified HYENZ in conjunction with a suitable pharmaceutical carrier may be administered to a subject to treat or prevent a disorder associated with decreased expression or activity of HYENZ including, but not limited to, those provided above.

5 In still another embodiment, an agonist which modulates the activity of HYENZ may be administered to a subject to treat or prevent a disorder associated with decreased expression or activity of HYENZ including, but not limited to, those listed above.

In a further embodiment, an antagonist of HYENZ may be administered to a subject to treat or prevent a disorder associated with increased expression or activity of HYENZ. Examples of such
10 disorders include, but are not limited to, those neurological disorders, immune system disorders, genetic disorders, and cell proliferation disorders, including cancer described above. In one aspect, an antibody which specifically binds HYENZ may be used directly as an antagonist or indirectly as a targeting or delivery mechanism for bringing a pharmaceutical agent to cells or tissues which express HYENZ.

In an additional embodiment, a vector expressing the complement of the polynucleotide
15 encoding HYENZ may be administered to a subject to treat or prevent a disorder associated with increased expression or activity of HYENZ including, but not limited to, those described above.

In other embodiments, any of the proteins, antagonists, antibodies, agonists, complementary sequences, or vectors of the invention may be administered in combination with other appropriate therapeutic agents. Selection of the appropriate agents for use in combination therapy may be made by
20 one of ordinary skill in the art, according to conventional pharmaceutical principles. The combination of therapeutic agents may act synergistically to effect the treatment or prevention of the various disorders described above. Using this approach, one may be able to achieve therapeutic efficacy with lower dosages of each agent, thus reducing the potential for adverse side effects.

An antagonist of HYENZ may be produced using methods which are generally known in the
25 art. In particular, purified HYENZ may be used to produce antibodies or to screen libraries of pharmaceutical agents to identify those which specifically bind HYENZ. Antibodies to HYENZ may also be generated using methods that are well known in the art. Such antibodies may include, but are not limited to, polyclonal, monoclonal, chimeric, and single chain antibodies, Fab fragments, and fragments produced by a Fab expression library. Neutralizing antibodies (i.e., those which inhibit
30 dimer formation) are generally preferred for therapeutic use.

For the production of antibodies, various hosts including goats, rabbits, rats, mice, humans, and others may be immunized by injection with HYENZ or with any fragment or oligopeptide thereof which has immunogenic properties. Depending on the host species, various adjuvants may be used to increase immunological response. Such adjuvants include, but are not limited to, Freund's, mineral gels

such as aluminum hydroxide, and surface active substances such as lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, KLH, and dinitrophenol. Among adjuvants used in humans, BCG (bacilli Calmette-Guerin) and Corynebacterium parvum are especially preferable.

It is preferred that the oligopeptides, peptides, or fragments used to induce antibodies to HYENZ have an amino acid sequence consisting of at least about 5 amino acids, and generally will consist of at least about 10 amino acids. It is also preferable that these oligopeptides, peptides, or fragments are identical to a portion of the amino acid sequence of the natural protein. Short stretches of HYENZ amino acids may be fused with those of another protein, such as KLH, and antibodies to the chimeric molecule may be produced.

Monoclonal antibodies to HYENZ may be prepared using any technique which provides for the production of antibody molecules by continuous cell lines in culture. These include, but are not limited to, the hybridoma technique, the human B-cell hybridoma technique, and the EBV-hybridoma technique. (See, e.g., Kohler, G. et al. (1975) Nature 256:495-497; Kozbor, D. et al. (1985) J. Immunol. Methods 81:31-42; Cote, R.J. et al. (1983) Proc. Natl. Acad. Sci. USA 80:2026-2030; and Cole, S.P. et al. (1984) Mol. Cell Biol. 62:109-120.)

In addition, techniques developed for the production of "chimeric antibodies," such as the splicing of mouse antibody genes to human antibody genes to obtain a molecule with appropriate antigen specificity and biological activity, can be used. (See, e.g., Morrison, S.L. et al. (1984) Proc. Natl. Acad. Sci. USA 81:6851-6855; Neuberger, M.S. et al. (1984) Nature 312:604-608; and Takeda, S. et al. (1985) Nature 314:452-454.) Alternatively, techniques described for the production of single chain antibodies may be adapted, using methods known in the art, to produce HYENZ-specific single chain antibodies. Antibodies with related specificity, but of distinct idiotypic composition, may be generated by chain shuffling from random combinatorial immunoglobulin libraries. (See, e.g., Burton, D.R. (1991) Proc. Natl. Acad. Sci. USA 88:10134-10137.)

Antibodies may also be produced by inducing in vivo production in the lymphocyte population or by screening immunoglobulin libraries or panels of highly specific binding reagents as disclosed in the literature. (See, e.g., Orlandi, R. et al. (1989) Proc. Natl. Acad. Sci. USA 86:3833-3837; Winter, G. et al. (1991) Nature 349:293-299.)

Antibody fragments which contain specific binding sites for HYENZ may also be generated. For example, such fragments include, but are not limited to, F(ab')₂ fragments produced by pepsin digestion of the antibody molecule and Fab fragments generated by reducing the disulfide bridges of the F(ab')₂ fragments. Alternatively, Fab expression libraries may be constructed to allow rapid and easy identification of monoclonal Fab fragments with the desired specificity. (See, e.g., Huse, W.D. et al. (1989) Science 246:1275-1281.)

Various immunoassays may be used for screening to identify antibodies having the desired specificity. Numerous protocols for competitive binding or immunoradiometric assays using either polyclonal or monoclonal antibodies with established specificities are well known in the art. Such immunoassays typically involve the measurement of complex formation between HYENZ and its specific antibody. A two-site, monoclonal-based immunoassay utilizing monoclonal antibodies reactive to two non-interfering HYENZ epitopes is generally used, but a competitive binding assay may also be employed (Pound, *supra*).

Various methods such as Scatchard analysis in conjunction with radioimmunoassay techniques may be used to assess the affinity of antibodies for HYENZ. Affinity is expressed as an association constant, K_a , which is defined as the molar concentration of HYENZ-antibody complex divided by the molar concentrations of free antigen and free antibody under equilibrium conditions. The K_a determined for a preparation of polyclonal antibodies, which are heterogeneous in their affinities for multiple HYENZ epitopes, represents the average affinity, or avidity, of the antibodies for HYENZ. The K_a determined for a preparation of monoclonal antibodies, which are monospecific for a particular HYENZ epitope, represents a true measure of affinity. High-affinity antibody preparations with K_a ranging from about 10^9 to 10^{12} L/mole are preferred for use in immunoassays in which the HYENZ-antibody complex must withstand rigorous manipulations. Low-affinity antibody preparations with K_a ranging from about 10^6 to 10^7 L/mole are preferred for use in immunopurification and similar procedures which ultimately require dissociation of HYENZ, preferably in active form, from the antibody (Catty, D. (1988) Antibodies, Volume I: A Practical Approach, IRL Press, Washington DC; Liddell, J.E. and A. Cryer (1991) A Practical Guide to Monoclonal Antibodies, John Wiley & Sons, New York NY).

The titer and avidity of polyclonal antibody preparations may be further evaluated to determine the quality and suitability of such preparations for certain downstream applications. For example, a polyclonal antibody preparation containing at least 1-2 mg specific antibody/ml, preferably 5-10 mg specific antibody/ml, is generally employed in procedures requiring precipitation of HYENZ-antibody complexes. Procedures for evaluating antibody specificity, titer, and avidity, and guidelines for antibody quality and usage in various applications, are generally available. (See, e.g., Catty, *supra*, and Coligan et al., *supra*.)

In another embodiment of the invention, the polynucleotides encoding HYENZ, or any fragment or complement thereof, may be used for therapeutic purposes. In one aspect, modifications of gene expression can be achieved by designing complementary sequences or antisense molecules (DNA, RNA, PNA, or modified oligonucleotides) to the coding or regulatory regions of the gene encoding HYENZ. Such technology is well known in the art, and antisense oligonucleotides or larger fragments can be

designed from various locations along the coding or control regions of sequences encoding HYENZ. (See, e.g., Agrawal, S., ed. (1996) Antisense Therapeutics, Humana Press Inc., Totawa NJ.)

In therapeutic use, any gene delivery system suitable for introduction of the antisense sequences into appropriate target cells can be used. Antisense sequences can be delivered intracellularly in the form of an expression plasmid which, upon transcription, produces a sequence complementary to at least a portion of the cellular sequence encoding the target protein. (See, e.g., Slater, J.E. et al. (1998) *J. Allergy Clin. Immunol.* 102(3):469-475; and Scanlon, K.J. et al. (1995) 9(13):1288-1296.) Antisense sequences can also be introduced intracellularly through the use of viral vectors, such as retrovirus and adeno-associated virus vectors. (See, e.g., Miller, A.D. (1990) *Blood* 76:271; Ausubel, supra; Uckert, W. and W. Walther (1994) *Pharmacol. Ther.* 63(3):323-347.) Other gene delivery mechanisms include liposome-derived systems, artificial viral envelopes, and other systems known in the art. (See, e.g., Rossi, J.J. (1995) *Br. Med. Bull.* 51(1):217-225; Boado, R.J. et al. (1998) *J. Pharm. Sci.* 87(11):1308-1315; and Morris, M.C. et al. (1997) *Nucleic Acids Res.* 25(14):2730-2736.)

In another embodiment of the invention, polynucleotides encoding HYENZ may be used for somatic or germline gene therapy. Gene therapy may be performed to (i) correct a genetic deficiency (e.g., in the cases of severe combined immunodeficiency (SCID)-X1 disease characterized by X-linked inheritance (Cavazzana-Calvo, M. et al. (2000) *Science* 288:669-672), severe combined immunodeficiency syndrome associated with an inherited adenosine deaminase (ADA) deficiency (Blaese, R.M. et al. (1995) *Science* 270:475-480; Bordignon, C. et al. (1995) *Science* 270:470-475), cystic fibrosis (Zabner, J. et al. (1993) *Cell* 75:207-216; Crystal, R.G. et al. (1995) *Hum. Gene Therapy* 6:643-666; Crystal, R.G. et al. (1995) *Hum. Gene Therapy* 6:667-703), thalassemias, familial hypercholesterolemia, and hemophilia resulting from Factor VIII or Factor IX deficiencies (Crystal, R.G. (1995) *Science* 270:404-410; Verma, I.M. and N. Somia (1997) *Nature* 389:239-242)), (ii) express a conditionally lethal gene product (e.g., in the case of cancers which result from unregulated cell proliferation), or (iii) express a protein which affords protection against intracellular parasites (e.g., against human retroviruses, such as human immunodeficiency virus (HIV) (Baltimore, D. (1988) *Nature* 335:395-396; Poeschla, E. et al. (1996) *Proc. Natl. Acad. Sci. USA.* 93:11395-11399), hepatitis B or C virus (HBV, HCV); fungal parasites, such as Candida albicans and Paracoccidioides brasiliensis; and protozoan parasites such as Plasmodium falciparum and Trypanosoma cruzi). In the case where a genetic deficiency in HYENZ expression or regulation causes disease, the expression of HYENZ from an appropriate population of transduced cells may alleviate the clinical manifestations caused by the genetic deficiency.

In a further embodiment of the invention, diseases or disorders caused by deficiencies in HYENZ are treated by constructing mammalian expression vectors encoding HYENZ and introducing

these vectors by mechanical means into HYENZ-deficient cells. Mechanical transfer technologies for use with cells in vivo or ex vitro include (i) direct DNA microinjection into individual cells, (ii) ballistic gold particle delivery, (iii) liposome-mediated transfection, (iv) receptor-mediated gene transfer, and (v) the use of DNA transposons (Morgan, R.A. and W.F. Anderson (1993) *Annu. Rev. Biochem.* 62:191-217; Ivics, Z. (1997) *Cell* 91:501-510; Boulay, J-L. and H. Récipon (1998) *Curr. Opin. Biotechnol.* 9:445-450).

Expression vectors that may be effective for the expression of HYENZ include, but are not limited to, the PCDNA 3.1, EPITAG, PRCCMV2, PREP, PVAX vectors (Invitrogen, Carlsbad CA), PCMV-SCRIPT, PCMV-TAG, PEGSH/PERV (Stratagene, La Jolla CA), and PTET-OFF, PTET-ON, PTRE2, PTRE2-LUC, PTK-HYG (Clontech, Palo Alto CA). HYENZ may be expressed using (i) a constitutively active promoter, (e.g., from cytomegalovirus (CMV), Rous sarcoma virus (RSV), SV40 virus, thymidine kinase (TK), or β -actin genes), (ii) an inducible promoter (e.g., the tetracycline-regulated promoter (Gossen, M. and H. Bujard (1992) *Proc. Natl. Acad. Sci. USA* 89:5547-5551; Gossen, M. et al. (1995) *Science* 268:1766-1769; Rossi, F.M.V. and H.M. Blau (1998) *Curr. Opin. Biotechnol.* 9:451-456), commercially available in the T-REX plasmid (Invitrogen)); the ecdysone-inducible promoter (available in the plasmids PVGRXR and PIND; Invitrogen); the FK506/rapamycin inducible promoter; or the RU486/mifepristone inducible promoter (Rossi, F.M.V. and H.M. Blau, supra), or (iii) a tissue-specific promoter or the native promoter of the endogenous gene encoding HYENZ from a normal individual.

Commercially available liposome transformation kits (e.g., the PERFECT LIPID TRANSFECTION KIT, available from Invitrogen) allow one with ordinary skill in the art to deliver polynucleotides to target cells in culture and require minimal effort to optimize experimental parameters. In the alternative, transformation is performed using the calcium phosphate method (Graham, F.L. and A.J. Eb (1973) *Virology* 52:456-467), or by electroporation (Neumann, E. et al. (1982) *EMBO J.* 1:841-845). The introduction of DNA to primary cells requires modification of these standardized mammalian transfection protocols.

In another embodiment of the invention, diseases or disorders caused by genetic defects with respect to HYENZ expression are treated by constructing a retrovirus vector consisting of (i) the polynucleotide encoding HYENZ under the control of an independent promoter or the retrovirus long terminal repeat (LTR) promoter, (ii) appropriate RNA packaging signals, and (iii) a Rev-responsive element (RRE) along with additional retrovirus *cis*-acting RNA sequences and coding sequences required for efficient vector propagation. Retrovirus vectors (e.g., PFB and PFBNEO) are commercially available (Stratagene) and are based on published data (Riviere, I. et al. (1995) *Proc. Natl. Acad. Sci. USA* 92:6733-6737), incorporated by reference herein. The vector is propagated in an

appropriate vector producing cell line (VPCL) that expresses an envelope gene with a tropism for receptors on the target cells or a promiscuous envelope protein such as VSVg (Armentano, D. et al. (1987) J. Virol. 61:1647-1650; Bender, M.A. et al. (1987) J. Virol. 61:1639-1646; Adam, M.A. and A.D. Miller (1988) J. Virol. 62:3802-3806; Dull, T. et al. (1998) J. Virol. 72:8463-8471; Zufferey, R. et al. (1998) J. Virol. 72:9873-9880). U.S. Patent Number 5,910,434 to Rigg ("Method for obtaining retrovirus packaging cell lines producing high transducing efficiency retroviral supernatant") discloses a method for obtaining retrovirus packaging cell lines and is hereby incorporated by reference.

Propagation of retrovirus vectors, transduction of a population of cells (e.g., CD4⁺ T-cells), and the return of transduced cells to a patient are procedures well known to persons skilled in the art of gene therapy and have been well documented (Ranga, U. et al. (1997) J. Virol. 71:7020-7029; Bauer, G. et al. (1997) Blood 89:2259-2267; Bonyhadi, M.L. (1997) J. Virol. 71:4707-4716; Ranga, U. et al. (1998) Proc. Natl. Acad. Sci. USA 95:1201-1206; Su, L. (1997) Blood 89:2283-2290).

In the alternative, an adenovirus-based gene therapy delivery system is used to deliver polynucleotides encoding HYENZ to cells which have one or more genetic abnormalities with respect to the expression of HYENZ. The construction and packaging of adenovirus-based vectors are well known to those with ordinary skill in the art. Replication defective adenovirus vectors have proven to be versatile for importing genes encoding immunoregulatory proteins into intact islets in the pancreas (Csete, M.E. et al. (1995) Transplantation 27:263-268). Potentially useful adenoviral vectors are described in U.S. Patent Number 5,707,618 to Armentano ("Adenovirus vectors for gene therapy"), hereby incorporated by reference. For adenoviral vectors, see also Antinozzi, P.A. et al. (1999) Annu. Rev. Nutr. 19:511-544; and Verma, I.M. and N. Somia (1997) Nature 18:389:239-242, both incorporated by reference herein.

In another alternative, a herpes-based, gene therapy delivery system is used to deliver polynucleotides encoding HYENZ to target cells which have one or more genetic abnormalities with respect to the expression of HYENZ. The use of herpes simplex virus (HSV)-based vectors may be especially valuable for introducing HYENZ to cells of the central nervous system, for which HSV has a tropism. The construction and packaging of herpes-based vectors are well known to those with ordinary skill in the art. A replication-competent herpes simplex virus (HSV) type 1-based vector has been used to deliver a reporter gene to the eyes of primates (Liu, X. et al. (1999) Exp. Eye Res. 169:385-395). The construction of a HSV-1 virus vector has also been disclosed in detail in U.S. Patent Number 5,804,413 to DeLuca ("Herpes simplex virus strains for gene transfer"), which is hereby incorporated by reference. U.S. Patent Number 5,804,413 teaches the use of recombinant HSV d92 which consists of a genome containing at least one exogenous gene to be transferred to a cell under the control of the appropriate promoter for purposes including human gene therapy. Also taught by this

patent are the construction and use of recombinant HSV strains deleted for ICP4, ICP27 and ICP22. For HSV vectors, see also Goins, W.F. et al. (1999) J. Virol. 73:519-532 and Xu, H. et al. (1994) Dev. Biol. 163:152-161, hereby incorporated by reference. The manipulation of cloned herpesvirus sequences, the generation of recombinant virus following the transfection of multiple plasmids containing different segments of the large herpesvirus genomes, the growth and propagation of herpesvirus, and the infection of cells with herpesvirus are techniques well known to those of ordinary skill in the art.

In another alternative, an alphavirus (positive, single-stranded RNA virus) vector is used to deliver polynucleotides encoding HYENZ to target cells. The biology of the prototypic alphavirus, Semliki Forest Virus (SFV), has been studied extensively and gene transfer vectors have been based on the SFV genome (Garoff, H. and K.-J. Li (1998) Curr. Opin. Biotechnol. 9:464-469). During alphavirus RNA replication, a subgenomic RNA is generated that normally encodes the viral capsid proteins. This subgenomic RNA replicates to higher levels than the full-length genomic RNA, resulting in the overproduction of capsid proteins relative to the viral proteins with enzymatic activity (e.g., protease and polymerase). Similarly, inserting the coding sequence for HYENZ into the alphavirus genome in place of the capsid-coding region results in the production of a large number of HYENZ-coding RNAs and the synthesis of high levels of HYENZ in vector transduced cells. While alphavirus infection is typically associated with cell lysis within a few days, the ability to establish a persistent infection in hamster normal kidney cells (BHK-21) with a variant of Sindbis virus (SIN) indicates that the lytic replication of alphaviruses can be altered to suit the needs of the gene therapy application (Dryga, S.A. et al. (1997) Virology 228:74-83). The wide host range of alphaviruses will allow the introduction of HYENZ into a variety of cell types. The specific transduction of a subset of cells in a population may require the sorting of cells prior to transduction. The methods of manipulating infectious cDNA clones of alphaviruses, performing alphavirus cDNA and RNA transfections, and performing alphavirus infections, are well known to those with ordinary skill in the art.

Oligonucleotides derived from the transcription initiation site, e.g., between about positions -10 and +10 from the start site, may also be employed to inhibit gene expression. Similarly, inhibition can be achieved using triple helix base-pairing methodology. Triple helix pairing is useful because it causes inhibition of the ability of the double helix to open sufficiently for the binding of polymerases, transcription factors, or regulatory molecules. Recent therapeutic advances using triplex DNA have been described in the literature. (See, e.g., Gee, J.E. et al. (1994) in Huber, B.E. and B.I. Carr, Molecular and Immunologic Approaches, Futura Publishing, Mt. Kisco NY, pp. 163-177.) A complementary sequence or antisense molecule may also be designed to block translation of mRNA by preventing the transcript from binding to ribosomes.

Ribozymes, enzymatic RNA molecules, may also be used to catalyze the specific cleavage of RNA. The mechanism of ribozyme action involves sequence-specific hybridization of the ribozyme molecule to complementary target RNA, followed by endonucleolytic cleavage. For example, engineered hammerhead motif ribozyme molecules may specifically and efficiently catalyze endonucleolytic cleavage of sequences encoding HYENZ.

Specific ribozyme cleavage sites within any potential RNA target are initially identified by scanning the target molecule for ribozyme cleavage sites, including the following sequences: GUA, GUU, and GUC. Once identified, short RNA sequences of between 15 and 20 ribonucleotides, corresponding to the region of the target gene containing the cleavage site, may be evaluated for secondary structural features which may render the oligonucleotide inoperable. The suitability of candidate targets may also be evaluated by testing accessibility to hybridization with complementary oligonucleotides using ribonuclease protection assays.

Complementary ribonucleic acid molecules and ribozymes of the invention may be prepared by any method known in the art for the synthesis of nucleic acid molecules. These include techniques for chemically synthesizing oligonucleotides such as solid phase phosphoramidite chemical synthesis. Alternatively, RNA molecules may be generated by *in vitro* and *in vivo* transcription of DNA sequences encoding HYENZ. Such DNA sequences may be incorporated into a wide variety of vectors with suitable RNA polymerase promoters such as T7 or SP6. Alternatively, these cDNA constructs that synthesize complementary RNA, constitutively or inducibly, can be introduced into cell lines, cells, or tissues.

RNA molecules may be modified to increase intracellular stability and half-life. Possible modifications include, but are not limited to, the addition of flanking sequences at the 5' and/or 3' ends of the molecule, or the use of phosphorothioate or 2' O-methyl rather than phosphodiesterase linkages within the backbone of the molecule. This concept is inherent in the production of PNAs and can be extended in all of these molecules by the inclusion of nontraditional bases such as inosine, queosine, and wybutosine, as well as acetyl-, methyl-, thio-, and similarly modified forms of adenine, cytidine, guanine, thymine, and uridine which are not as easily recognized by endogenous endonucleases.

An additional embodiment of the invention encompasses a method for screening for a compound which is effective in altering expression of a polynucleotide encoding HYENZ.

Compounds which may be effective in altering expression of a specific polynucleotide may include, but are not limited to, oligonucleotides, antisense oligonucleotides, triple helix-forming oligonucleotides, transcription factors and other polypeptide transcriptional regulators, and non-macromolecular chemical entities which are capable of interacting with specific polynucleotide sequences. Effective compounds may alter polynucleotide expression by acting as either inhibitors or promoters of polynucleotide expression. Thus, in the treatment of disorders associated with increased

HYENZ expression or activity, a compound which specifically inhibits expression of the polynucleotide encoding HYENZ may be therapeutically useful, and in the treatment of disorders associated with decreased HYENZ expression or activity, a compound which specifically promotes expression of the polynucleotide encoding HYENZ may be therapeutically useful.

5 At least one, and up to a plurality, of test compounds may be screened for effectiveness in altering expression of a specific polynucleotide. A test compound may be obtained by any method commonly known in the art, including chemical modification of a compound known to be effective in altering polynucleotide expression; selection from an existing, commercially-available or proprietary library of naturally-occurring or non-natural chemical compounds; rational design of a compound
10 based on chemical and/or structural properties of the target polynucleotide; and selection from a library of chemical compounds created combinatorially or randomly. A sample comprising a polynucleotide encoding HYENZ is exposed to at least one test compound thus obtained. The sample may comprise, for example, an intact or permeabilized cell, or an in vitro cell-free or reconstituted biochemical system. Alterations in the expression of a polynucleotide encoding HYENZ are assayed
15 by any method commonly known in the art. Typically, the expression of a specific nucleotide is detected by hybridization with a probe having a nucleotide sequence complementary to the sequence of the polynucleotide encoding HYENZ. The amount of hybridization may be quantified, thus forming the basis for a comparison of the expression of the polynucleotide both with and without exposure to one or more test compounds. Detection of a change in the expression of a polynucleotide
20 exposed to a test compound indicates that the test compound is effective in altering the expression of the polynucleotide. A screen for a compound effective in altering expression of a specific polynucleotide can be carried out, for example, using a Schizosaccharomyces pombe gene expression system (Atkins, D. et al. (1999) U.S. Patent No. 5,932,435; Arndt, G.M. et al. (2000) Nucleic Acids Res. 28:E15) or a human cell line such as HeLa cell (Clarke, M.L. et al. (2000) Biochem. Biophys. Res. Commun. 268:8-13). A particular embodiment of the present invention involves screening a
25 combinatorial library of oligonucleotides (such as deoxyribonucleotides, ribonucleotides, peptide nucleic acids, and modified oligonucleotides) for antisense activity against a specific polynucleotide sequence (Bruce, T.W. et al. (1997) U.S. Patent No. 5,686,242; Bruce, T.W. et al. (2000) U.S. Patent No. 6,022,691).

30 Many methods for introducing vectors into cells or tissues are available and equally suitable for use in vivo, in vitro, and ex vivo. For ex vivo therapy, vectors may be introduced into stem cells taken from the patient and clonally propagated for autologous transplant back into that same patient. Delivery by transfection, by liposome injections, or by polycationic amino polymers may be achieved using methods which are well known in the art. (See, e.g., Goldman, C.K. et al. (1997) Nat.
35 Biotechnol. 15:462-466.)

Any of the therapeutic methods described above may be applied to any subject in need of such therapy, including, for example, mammals such as humans, dogs, cats, cows, horses, rabbits, and monkeys.

5 An additional embodiment of the invention relates to the administration of a composition which generally comprises an active ingredient formulated with a pharmaceutically acceptable excipient. Excipients may include, for example, sugars, starches, celluloses, gums, and proteins. Various formulations are commonly known and are thoroughly discussed in the latest edition of Remington's Pharmaceutical Sciences (Maack Publishing, Easton PA). Such compositions may consist of HYENZ, antibodies to HYENZ, and mimetics, agonists, antagonists, or inhibitors of HYENZ.

10 The compositions utilized in this invention may be administered by any number of routes including, but not limited to, oral, intravenous, intramuscular, intra-arterial, intramedullary, intrathecal, intraventricular, pulmonary, transdermal, subcutaneous, intraperitoneal, intranasal, enteral, topical, sublingual, or rectal means.

Compositions for pulmonary administration may be prepared in liquid or dry powder form.
15 These compositions are generally aerosolized immediately prior to inhalation by the patient. In the case of small molecules (e.g. traditional low molecular weight organic drugs), aerosol delivery of fast-acting formulations is well-known in the art. In the case of macromolecules (e.g. larger peptides and proteins), recent developments in the field of pulmonary delivery via the alveolar region of the lung have enabled the practical delivery of drugs such as insulin to blood circulation (see, e.g., Patton, J.S. et al., U.S.
20 Patent No. 5,997,848). Pulmonary delivery has the advantage of administration without needle injection, and obviates the need for potentially toxic penetration enhancers.

Compositions suitable for use in the invention include compositions wherein the active ingredients are contained in an effective amount to achieve the intended purpose. The determination of an effective dose is well within the capability of those skilled in the art.

25 Specialized forms of compositions may be prepared for direct intracellular delivery of macromolecules comprising HYENZ or fragments thereof. For example, liposome preparations containing a cell-impermeable macromolecule may promote cell fusion and intracellular delivery of the macromolecule. Alternatively, HYENZ or a fragment thereof may be joined to a short cationic N-terminal portion from the HIV Tat-1 protein. Fusion proteins thus generated have been found to
30 transduce into the cells of all tissues, including the brain, in a mouse model system (Schwarze, S.R. et al. (1999) Science 285:1569-1572).

For any compound, the therapeutically effective dose can be estimated initially either in cell culture assays, e.g., of neoplastic cells, or in animal models such as mice, rats, rabbits, dogs, monkeys, or pigs. An animal model may also be used to determine the appropriate concentration range and route

of administration. Such information can then be used to determine useful doses and routes for administration in humans.

A therapeutically effective dose refers to that amount of active ingredient, for example HYENZ or fragments thereof, antibodies of HYENZ, and agonists, antagonists or inhibitors of HYENZ, which ameliorates the symptoms or condition. Therapeutic efficacy and toxicity may be determined by standard pharmaceutical procedures in cell cultures or with experimental animals, such as by calculating the ED_{50} (the dose therapeutically effective in 50% of the population) or LD_{50} (the dose lethal to 50% of the population) statistics. The dose ratio of toxic to therapeutic effects is the therapeutic index, which can be expressed as the LD_{50}/ED_{50} ratio. Compositions which exhibit large therapeutic indices are preferred. The data obtained from cell culture assays and animal studies are used to formulate a range of dosage for human use. The dosage contained in such compositions is preferably within a range of circulating concentrations that includes the ED_{50} with little or no toxicity. The dosage varies within this range depending upon the dosage form employed, the sensitivity of the patient, and the route of administration.

The exact dosage will be determined by the practitioner, in light of factors related to the subject requiring treatment. Dosage and administration are adjusted to provide sufficient levels of the active moiety or to maintain the desired effect. Factors which may be taken into account include the severity of the disease state, the general health of the subject, the age, weight, and gender of the subject, time and frequency of administration, drug combination(s), reaction sensitivities, and response to therapy. Long-acting compositions may be administered every 3 to 4 days, every week, or biweekly depending on the half-life and clearance rate of the particular formulation.

Normal dosage amounts may vary from about 0.1 μg to 100,000 μg , up to a total dose of about 1 gram, depending upon the route of administration. Guidance as to particular dosages and methods of delivery is provided in the literature and generally available to practitioners in the art.

Those skilled in the art will employ different formulations for nucleotides than for proteins or their inhibitors. Similarly, delivery of polynucleotides or polypeptides will be specific to particular cells, conditions, locations, etc.

DIAGNOSTICS

In another embodiment, antibodies which specifically bind HYENZ may be used for the diagnosis of disorders characterized by expression of HYENZ, or in assays to monitor patients being treated with HYENZ or agonists, antagonists, or inhibitors of HYENZ. Antibodies useful for diagnostic purposes may be prepared in the same manner as described above for therapeutics. Diagnostic assays for HYENZ include methods which utilize the antibody and a label to detect HYENZ in human body fluids or in extracts of cells or tissues. The antibodies may be used with or without

modification, and may be labeled by covalent or non-covalent attachment of a reporter molecule. A wide variety of reporter molecules, several of which are described above, are known in the art and may be used.

5 A variety of protocols for measuring HYENZ, including ELISAs, RIAs, and FACS, are known in the art and provide a basis for diagnosing altered or abnormal levels of HYENZ expression. Normal or standard values for HYENZ expression are established by combining body fluids or cell extracts taken from normal mammalian subjects, for example, human subjects, with antibody to HYENZ under conditions suitable for complex formation. The amount of standard complex formation may be quantitated by various methods, such as photometric means. Quantities of HYENZ expressed in
10 subject, control, and disease samples from biopsied tissues are compared with the standard values. Deviation between standard and subject values establishes the parameters for diagnosing disease.

In another embodiment of the invention, the polynucleotides encoding HYENZ may be used for diagnostic purposes. The polynucleotides which may be used include oligonucleotide sequences, complementary RNA and DNA molecules, and PNAs. The polynucleotides may be used to detect and
15 quantify gene expression in biopsied tissues in which expression of HYENZ may be correlated with disease. The diagnostic assay may be used to determine absence, presence, and excess expression of HYENZ, and to monitor regulation of HYENZ levels during therapeutic intervention.

In one aspect, hybridization with PCR probes which are capable of detecting polynucleotide sequences, including genomic sequences, encoding HYENZ or closely related molecules may be used to
20 identify nucleic acid sequences which encode HYENZ. The specificity of the probe, whether it is made from a highly specific region, e.g., the 5' regulatory region, or from a less specific region, e.g., a conserved motif, and the stringency of the hybridization or amplification will determine whether the probe identifies only naturally occurring sequences encoding HYENZ, allelic variants, or related sequences.

25 Probes may also be used for the detection of related sequences, and may have at least 50% sequence identity to any of the HYENZ encoding sequences. The hybridization probes of the subject invention may be DNA or RNA and may be derived from the sequence of SEQ ID NO:15-28 or from genomic sequences including promoters, enhancers, and introns of the HYENZ gene.

Means for producing specific hybridization probes for DNAs encoding HYENZ include the
30 cloning of polynucleotide sequences encoding HYENZ or HYENZ derivatives into vectors for the production of mRNA probes. Such vectors are known in the art, are commercially available, and may be used to synthesize RNA probes in vitro by means of the addition of the appropriate RNA polymerases and the appropriate labeled nucleotides. Hybridization probes may be labeled by a variety of reporter groups, for example, by radionuclides such as ³²P or ³⁵S, or by enzymatic labels, such as

alkaline phosphatase coupled to the probe via avidin/biotin coupling systems, and the like.

Polynucleotide sequences encoding HYENZ may be used for the diagnosis of disorders associated with expression of HYENZ. Examples of such disorders include, but are not limited to, a neurological disorder, such as epilepsy, ischemic cerebrovascular disease, stroke, cerebral neoplasms, Alzheimer's disease, Pick's disease, Huntington's disease, dementia, Parkinson's disease and other
5 extrapyramidal disorders, amyotrophic lateral sclerosis and other motor neuron disorders, progressive neural muscular atrophy, retinitis pigmentosa, hereditary ataxias, multiple sclerosis and other demyelinating diseases, bacterial and viral meningitis, brain abscess, subdural empyema, epidural abscess, suppurative intracranial thrombophlebitis, myelitis and radiculitis, viral central nervous system
10 disease; prion diseases including kuru, Creutzfeldt-Jakob disease, and Gerstmann-Straussler-Scheinker syndrome; fatal familial insomnia, nutritional and metabolic diseases of the nervous system, neurofibromatosis, tuberous sclerosis, cerebelloretinal hemangioblastomatosis, encephalotrigeminal syndrome, mental retardation and other developmental disorder of the central nervous system, cerebral palsy, a neuroskeletal disorder, an autonomic nervous system disorder, a cranial nerve disorder, a spinal
15 cord disease, muscular dystrophy and other neuromuscular disorder, a peripheral nervous system disorder, dermatomyositis and polymyositis; inherited, metabolic, endocrine, and toxic myopathy; myasthenia gravis, periodic paralysis; a mental disorder including mood, anxiety, and schizophrenic disorders; seasonal affective disorder (SAD); akathisia, amnesia, catatonia, diabetic neuropathy, tardive dyskinesia, dystonias, paranoid psychoses, postherpetic neuralgia, and Tourette's disorder; an
20 immune system disorder, such as inflammation, actinic keratosis, acquired immunodeficiency syndrome (AIDS), Addison's disease, adult respiratory distress syndrome, allergies, ankylosing spondylitis, amyloidosis, anemia, arteriosclerosis, asthma, atherosclerosis, autoimmune hemolytic anemia, autoimmune thyroiditis, bronchitis, bursitis, cholecystitis, cirrhosis, contact dermatitis, Crohn's disease, atopic dermatitis, dermatomyositis, diabetes mellitus, emphysema, erythroblastosis
25 fetalis, erythema nodosum, atrophic gastritis, glomerulonephritis, Goodpasture's syndrome, gout, Graves' disease, Hashimoto's thyroiditis, paroxysmal nocturnal hemoglobinuria, hepatitis, hypereosinophilia, irritable bowel syndrome, episodic lymphopenia with lymphocytotoxins, mixed connective tissue disease (MCTD), multiple sclerosis, myasthenia gravis, myocardial or pericardial inflammation, myofibrosis, osteoarthritis, osteoporosis, pancreatitis, polycythemia vera,
30 polymyositis, psoriasis, Reiter's syndrome, rheumatoid arthritis, scleroderma, Sjögren's syndrome, systemic anaphylaxis, systemic lupus erythematosus, systemic sclerosis, primary thrombocythemia, thrombocytopenic purpura, ulcerative colitis, uveitis, Werner syndrome, complications of cancer, hemodialysis, and extracorporeal circulation, trauma, and hematopoietic cancer including lymphoma, leukemia, and myeloma; a genetic disorder, such as GM1-gangliosidosis, Niemann-Pick disease,
35 adrenoleukodystrophy, Alport's syndrome, choroideremia, Duchenne and Becker muscular dystrophy,

Down's syndrome, cystic fibrosis, chronic granulomatous disease, Gaucher's disease, Huntington's chorea, Marfan's syndrome, muscular dystrophy, myotonic dystrophy, pycnodysostosis, Refsum's syndrome, retinoblastoma, sickle cell anemia, thalassemia, Werner syndrome, von Willebrand's disease, von Hippel-Lindau syndrome, Wilms' tumor, Zellweger syndrome, peroxisomal acyl-CoA oxidase deficiency, peroxisomal thiolase deficiency, peroxisomal bifunctional protein deficiency, mitochondrial carnitine palmitoyl transferase and carnitine deficiency, mitochondrial very-long-chain acyl-CoA dehydrogenase deficiency, mitochondrial medium-chain acyl-CoA dehydrogenase deficiency, mitochondrial short-chain acyl-CoA dehydrogenase deficiency, mitochondrial electron transport flavoprotein and electron transport flavoprotein:ubiquinone oxidoreductase deficiency, mitochondrial trifunctional protein deficiency, and mitochondrial short-chain 3-hydroxyacyl-CoA dehydrogenase deficiency; and a cell proliferation disorder, such as actinic keratosis, arteriosclerosis, atherosclerosis, bursitis, cirrhosis, hepatitis, mixed connective tissue disease (MCTD), myelofibrosis, paroxysmal nocturnal hemoglobinuria, polycythemia vera, psoriasis, primary thrombocythemia, and cancers including adenocarcinoma, leukemia, lymphoma, melanoma, myeloma, sarcoma, teratocarcinoma, and, in particular, a cancer of the adrenal gland, bladder, bone, bone marrow, brain, breast, cervix, gall bladder, ganglia, gastrointestinal tract, heart, kidney, liver, lung, muscle, ovary, pancreas, parathyroid, penis, prostate, salivary glands, skin, spleen, testis, thymus, thyroid, and uterus. The polynucleotide sequences encoding HYENZ may be used in Southern or northern analysis, dot blot, or other membrane-based technologies; in PCR technologies; in dipstick, pin, and multiformat ELISA-like assays; and in microarrays utilizing fluids or tissues from patients to detect altered HYENZ expression. Such qualitative or quantitative methods are well known in the art.

In a particular aspect, the nucleotide sequences encoding HYENZ may be useful in assays that detect the presence of associated disorders, particularly those mentioned above. The nucleotide sequences encoding HYENZ may be labeled by standard methods and added to a fluid or tissue sample from a patient under conditions suitable for the formation of hybridization complexes. After a suitable incubation period, the sample is washed and the signal is quantified and compared with a standard value. If the amount of signal in the patient sample is significantly altered in comparison to a control sample then the presence of altered levels of nucleotide sequences encoding HYENZ in the sample indicates the presence of the associated disorder. Such assays may also be used to evaluate the efficacy of a particular therapeutic treatment regimen in animal studies, in clinical trials, or to monitor the treatment of an individual patient.

In order to provide a basis for the diagnosis of a disorder associated with expression of HYENZ, a normal or standard profile for expression is established. This may be accomplished by combining body fluids or cell extracts taken from normal subjects, either animal or human, with a sequence, or a fragment thereof, encoding HYENZ, under conditions suitable for hybridization or

amplification. Standard hybridization may be quantified by comparing the values obtained from normal subjects with values from an experiment in which a known amount of a substantially purified polynucleotide is used. Standard values obtained in this manner may be compared with values obtained from samples from patients who are symptomatic for a disorder. Deviation from standard values is used to establish the presence of a disorder.

Once the presence of a disorder is established and a treatment protocol is initiated, hybridization assays may be repeated on a regular basis to determine if the level of expression in the patient begins to approximate that which is observed in the normal subject. The results obtained from successive assays may be used to show the efficacy of treatment over a period ranging from several days to months.

With respect to cancer, the presence of an abnormal amount of transcript (either under- or overexpressed) in biopsied tissue from an individual may indicate a predisposition for the development of the disease, or may provide a means for detecting the disease prior to the appearance of actual clinical symptoms. A more definitive diagnosis of this type may allow health professionals to employ preventative measures or aggressive treatment earlier thereby preventing the development or further progression of the cancer.

Additional diagnostic uses for oligonucleotides designed from the sequences encoding HYENZ may involve the use of PCR. These oligomers may be chemically synthesized, generated enzymatically, or produced in vitro. Oligomers will preferably contain a fragment of a polynucleotide encoding HYENZ, or a fragment of a polynucleotide complementary to the polynucleotide encoding HYENZ, and will be employed under optimized conditions for identification of a specific gene or condition. Oligomers may also be employed under less stringent conditions for detection or quantification of closely related DNA or RNA sequences.

In a particular aspect, oligonucleotide primers derived from the polynucleotide sequences encoding HYENZ may be used to detect single nucleotide polymorphisms (SNPs). SNPs are substitutions, insertions and deletions that are a frequent cause of inherited or acquired genetic disease in humans. Methods of SNP detection include, but are not limited to, single-stranded conformation polymorphism (SSCP) and fluorescent SSCP (fSSCP) methods. In SSCP, oligonucleotide primers derived from the polynucleotide sequences encoding HYENZ are used to amplify DNA using the polymerase chain reaction (PCR). The DNA may be derived, for example, from diseased or normal tissue, biopsy samples, bodily fluids, and the like. SNPs in the DNA cause differences in the secondary and tertiary structures of PCR products in single-stranded form, and these differences are detectable using gel electrophoresis in non-denaturing gels. In fSSCP, the oligonucleotide primers are fluorescently labeled, which allows detection of the amplimers in high-throughput equipment such as

DNA sequencing machines. Additionally, sequence database analysis methods, termed *in silico* SNP (isSNP), are capable of identifying polymorphisms by comparing the sequence of individual overlapping DNA fragments which assemble into a common consensus sequence. These computer-based methods filter out sequence variations due to laboratory preparation of DNA and sequencing errors using statistical models and automated analyses of DNA sequence chromatograms. In the alternative, SNPs may be detected and characterized by mass spectrometry using, for example, the high throughput MASSARRAY system (Sequenom, Inc., San Diego CA).

Methods which may also be used to quantify the expression of HYENZ include radiolabeling or biotinylating nucleotides, coamplification of a control nucleic acid, and interpolating results from standard curves. (See, e.g., Melby, P.C. et al. (1993) *J. Immunol. Methods* 159:235-244; Duplaa, C. et al. (1993) *Anal. Biochem.* 212:229-236.) The speed of quantitation of multiple samples may be accelerated by running the assay in a high-throughput format where the oligomer or polynucleotide of interest is presented in various dilutions and a spectrophotometric or colorimetric response gives rapid quantitation.

In further embodiments, oligonucleotides or longer fragments derived from any of the polynucleotide sequences described herein may be used as elements on a microarray. The microarray can be used in transcript imaging techniques which monitor the relative expression levels of large numbers of genes simultaneously as described in Seilhamer, J.J. et al., "Comparative Gene Transcript Analysis," U.S. Patent No. 5,840,484, incorporated herein by reference. The microarray may also be used to identify genetic variants, mutations, and polymorphisms. This information may be used to determine gene function, to understand the genetic basis of a disorder, to diagnose a disorder, to monitor progression/regression of disease as a function of gene expression, and to develop and monitor the activities of therapeutic agents in the treatment of disease. In particular, this information may be used to develop a pharmacogenomic profile of a patient in order to select the most appropriate and effective treatment regimen for that patient. For example, therapeutic agents which are highly effective and display the fewest side effects may be selected for a patient based on his/her pharmacogenomic profile.

In another embodiment, antibodies specific for HYENZ, or HYENZ or fragments thereof may be used as elements on a microarray. The microarray may be used to monitor or measure protein-protein interactions, drug-target interactions, and gene expression profiles, as described above.

A particular embodiment relates to the use of the polynucleotides of the present invention to generate a transcript image of a tissue or cell type. A transcript image represents the global pattern of gene expression by a particular tissue or cell type. Global gene expression patterns are analyzed by quantifying the number of expressed genes and their relative abundance under given conditions and at a given time. (See Seilhamer et al., "Comparative Gene Transcript Analysis," U.S. Patent Number

5,840,484, expressly incorporated by reference herein.) Thus a transcript image may be generated by hybridizing the polynucleotides of the present invention or their complements to the totality of transcripts or reverse transcripts of a particular tissue or cell type. In one embodiment, the hybridization takes place in high-throughput format, wherein the polynucleotides of the present invention or their complements comprise a subset of a plurality of elements on a microarray. The resultant transcript image would provide a profile of gene activity.

Transcript images may be generated using transcripts isolated from tissues, cell lines, biopsies, or other biological samples. The transcript image may thus reflect gene expression in vivo, as in the case of a tissue or biopsy sample, or in vitro, as in the case of a cell line.

Transcript images which profile the expression of the polynucleotides of the present invention may also be used in conjunction with in vitro model systems and preclinical evaluation of pharmaceuticals, as well as toxicological testing of industrial and naturally-occurring environmental compounds. All compounds induce characteristic gene expression patterns, frequently termed molecular fingerprints or toxicant signatures, which are indicative of mechanisms of action and toxicity (Nuwaysir, E.F. et al. (1999) Mol. Carcinog. 24:153-159; Steiner, S. and N.L. Anderson (2000) Toxicol. Lett. 112-113:467-471, expressly incorporated by reference herein). If a test compound has a signature similar to that of a compound with known toxicity, it is likely to share those toxic properties. These fingerprints or signatures are most useful and refined when they contain expression information from a large number of genes and gene families. Ideally, a genome-wide measurement of expression provides the highest quality signature. Even genes whose expression is not altered by any tested compounds are important as well, as the levels of expression of these genes are used to normalize the rest of the expression data. The normalization procedure is useful for comparison of expression data after treatment with different compounds. While the assignment of gene function to elements of a toxicant signature aids in interpretation of toxicity mechanisms, knowledge of gene function is not necessary for the statistical matching of signatures which leads to prediction of toxicity. (See, for example, Press Release 00-02 from the National Institute of Environmental Health Sciences, released February 29, 2000, available at <http://www.niehs.nih.gov/oc/news/toxchip.htm>.) Therefore, it is important and desirable in toxicological screening using toxicant signatures to include all expressed gene sequences.

In one embodiment, the toxicity of a test compound is assessed by treating a biological sample containing nucleic acids with the test compound. Nucleic acids that are expressed in the treated biological sample are hybridized with one or more probes specific to the polynucleotides of the present invention, so that transcript levels corresponding to the polynucleotides of the present invention may be quantified. The transcript levels in the treated biological sample are compared with levels in an untreated biological sample. Differences in the transcript levels between the two samples

are indicative of a toxic response caused by the test compound in the treated sample.

Another particular embodiment relates to the use of the polypeptide sequences of the present invention to analyze the proteome of a tissue or cell type. The term proteome refers to the global pattern of protein expression in a particular tissue or cell type. Each protein component of a proteome can be subjected individually to further analysis. Proteome expression patterns, or profiles, are analyzed by quantifying the number of expressed proteins and their relative abundance under given conditions and at a given time. A profile of a cell's proteome may thus be generated by separating and analyzing the polypeptides of a particular tissue or cell type. In one embodiment, the separation is achieved using two-dimensional gel electrophoresis, in which proteins from a sample are separated by isoelectric focusing in the first dimension, and then according to molecular weight by sodium dodecyl sulfate slab gel electrophoresis in the second dimension (Steiner and Anderson, *supra*). The proteins are visualized in the gel as discrete and uniquely positioned spots, typically by staining the gel with an agent such as Coomassie Blue or silver or fluorescent stains. The optical density of each protein spot is generally proportional to the level of the protein in the sample. The optical densities of equivalently positioned protein spots from different samples, for example, from biological samples either treated or untreated with a test compound or therapeutic agent, are compared to identify any changes in protein spot density related to the treatment. The proteins in the spots are partially sequenced using, for example, standard methods employing chemical or enzymatic cleavage followed by mass spectrometry. The identity of the protein in a spot may be determined by comparing its partial sequence, preferably of at least 5 contiguous amino acid residues, to the polypeptide sequences of the present invention. In some cases, further sequence data may be obtained for definitive protein identification.

A proteomic profile may also be generated using antibodies specific for HYENZ to quantify the levels of HYENZ expression. In one embodiment, the antibodies are used as elements on a microarray, and protein expression levels are quantified by exposing the microarray to the sample and detecting the levels of protein bound to each array element (Lueking, A. et al. (1999) *Anal. Biochem.* 270:103-111; Mendoze, L.G. et al. (1999) *Biotechniques* 27:778-788). Detection may be performed by a variety of methods known in the art, for example, by reacting the proteins in the sample with a thiol- or amino-reactive fluorescent compound and detecting the amount of fluorescence bound at each array element.

Toxicant signatures at the proteome level are also useful for toxicological screening, and should be analyzed in parallel with toxicant signatures at the transcript level. There is a poor correlation between transcript and protein abundances for some proteins in some tissues (Anderson, N.L. and J. Seilhamer (1997) *Electrophoresis* 18:533-537), so proteome toxicant signatures may be useful in the analysis of compounds which do not significantly affect the transcript image, but which alter the proteomic profile. In addition, the analysis of transcripts in body fluids is difficult, due to rapid

degradation of mRNA, so proteomic profiling may be more reliable and informative in such cases.

In another embodiment, the toxicity of a test compound is assessed by treating a biological sample containing proteins with the test compound. Proteins that are expressed in the treated biological sample are separated so that the amount of each protein can be quantified. The amount of each protein is compared to the amount of the corresponding protein in an untreated biological sample. A difference in the amount of protein between the two samples is indicative of a toxic response to the test compound in the treated sample. Individual proteins are identified by sequencing the amino acid residues of the individual proteins and comparing these partial sequences to the polypeptides of the present invention.

In another embodiment, the toxicity of a test compound is assessed by treating a biological sample containing proteins with the test compound. Proteins from the biological sample are incubated with antibodies specific to the polypeptides of the present invention. The amount of protein recognized by the antibodies is quantified. The amount of protein in the treated biological sample is compared with the amount in an untreated biological sample. A difference in the amount of protein between the two samples is indicative of a toxic response to the test compound in the treated sample.

Microarrays may be prepared, used, and analyzed using methods known in the art. (See, e.g., Brennan, T.M. et al. (1995) U.S. Patent No. 5,474,796; Schena, M. et al. (1996) Proc. Natl. Acad. Sci. USA 93:10614-10619; Baldeschweiler et al. (1995) PCT application WO95/251116; Shalon, D. et al. (1995) PCT application WO95/35505; Heller, R.A. et al. (1997) Proc. Natl. Acad. Sci. USA 94:2150-2155; and Heller, M.J. et al. (1997) U.S. Patent No. 5,605,662.) Various types of microarrays are well known and thoroughly described in DNA Microarrays: A Practical Approach, M. Schena, ed. (1999) Oxford University Press, London, hereby expressly incorporated by reference.

In another embodiment of the invention, nucleic acid sequences encoding HYENZ may be used to generate hybridization probes useful in mapping the naturally occurring genomic sequence. Either coding or noncoding sequences may be used, and in some instances, noncoding sequences may be preferable over coding sequences. For example, conservation of a coding sequence among members of a multi-gene family may potentially cause undesired cross hybridization during chromosomal mapping. The sequences may be mapped to a particular chromosome, to a specific region of a chromosome, or to artificial chromosome constructions, e.g., human artificial chromosomes (HACs), yeast artificial chromosomes (YACs), bacterial artificial chromosomes (BACs), bacterial P1 constructions, or single chromosome cDNA libraries. (See, e.g., Harrington, J.J. et al. (1997) Nat. Genet. 15:345-355; Price, C.M. (1993) Blood Rev. 7:127-134; and Trask, B.J. (1991) Trends Genet. 7:149-154.) Once mapped, the nucleic acid sequences of the invention may be used to develop genetic linkage maps, for example, which correlate the inheritance of a disease state with the inheritance of a particular chromosome region or restriction fragment length polymorphism (RFLP). (See, e.g., Lander, E.S. and D. Botstein (1986) Proc. Natl. Acad. Sci. USA 83:7353-7357.)

Fluorescent in situ hybridization (FISH) may be correlated with other physical and genetic map data. (See, e.g., Heinz-Ulrich, et al. (1995) in Meyers, supra, pp. 965-968.) Examples of genetic map data can be found in various scientific journals or at the Online Mendelian Inheritance in Man (OMIM) World Wide Web site. Correlation between the location of the gene encoding HYENZ on a physical map and a specific disorder, or a predisposition to a specific disorder, may help define the region of DNA associated with that disorder and thus may further positional cloning efforts.

In situ hybridization of chromosomal preparations and physical mapping techniques, such as linkage analysis using established chromosomal markers, may be used for extending genetic maps. Often the placement of a gene on the chromosome of another mammalian species, such as mouse, may reveal associated markers even if the exact chromosomal locus is not known. This information is valuable to investigators searching for disease genes using positional cloning or other gene discovery techniques. Once the gene or genes responsible for a disease or syndrome have been crudely localized by genetic linkage to a particular genomic region, e.g., ataxia-telangiectasia to 11q22-23, any sequences mapping to that area may represent associated or regulatory genes for further investigation. (See, e.g., Gatti, R.A. et al. (1988) Nature 336:577-580.) The nucleotide sequence of the instant invention may also be used to detect differences in the chromosomal location due to translocation, inversion, etc., among normal, carrier, or affected individuals.

In another embodiment of the invention, HYENZ, its catalytic or immunogenic fragments, or oligopeptides thereof can be used for screening libraries of compounds in any of a variety of drug screening techniques. The fragment employed in such screening may be free in solution, affixed to a solid support, borne on a cell surface, or located intracellularly. The formation of binding complexes between HYENZ and the agent being tested may be measured.

Another technique for drug screening provides for high throughput screening of compounds having suitable binding affinity to the protein of interest. (See, e.g., Geysen, et al. (1984) PCT application WO84/03564.) In this method, large numbers of different small test compounds are synthesized on a solid substrate. The test compounds are reacted with HYENZ, or fragments thereof, and washed. Bound HYENZ is then detected by methods well known in the art. Purified HYENZ can also be coated directly onto plates for use in the aforementioned drug screening techniques. Alternatively, non-neutralizing antibodies can be used to capture the peptide and immobilize it on a solid support.

In another embodiment, one may use competitive drug screening assays in which neutralizing antibodies capable of binding HYENZ specifically compete with a test compound for binding HYENZ. In this manner, antibodies can be used to detect the presence of any peptide which shares one or more antigenic determinants with HYENZ.

In additional embodiments, the nucleotide sequences which encode HYENZ may be used in any molecular biology techniques that have yet to be developed, provided the new techniques rely on properties of nucleotide sequences that are currently known, including, but not limited to, such properties as the triplet genetic code and specific base pair interactions.

Without further elaboration, it is believed that one skilled in the art can, using the preceding description, utilize the present invention to its fullest extent. The following preferred specific embodiments are, therefore, to be construed as merely illustrative, and not limitative of the remainder of the disclosure in any way whatsoever.

The disclosures of all patents, applications and publications, mentioned above and below, in particular U.S. Ser. No. 60/151,819, are hereby expressly incorporated by reference.

EXAMPLES

I. Construction of cDNA Libraries

RNA was purchased from Clontech or isolated from tissues described in Table 4. Some tissues were homogenized and lysed in guanidinium isothiocyanate, while others were homogenized and lysed in phenol or in a suitable mixture of denaturants, such as TRIZOL (Life Technologies), a monophasic solution of phenol and guanidine isothiocyanate. The resulting lysates were centrifuged over CsCl cushions or extracted with chloroform. RNA was precipitated from the lysates with either isopropanol or sodium acetate and ethanol, or by other routine methods.

Phenol extraction and precipitation of RNA were repeated as necessary to increase RNA purity. In some cases, RNA was treated with DNase. For most libraries, poly(A⁺) RNA was isolated using oligo d(T)-coupled paramagnetic particles (Promega), OLIGOTEX latex particles (QIAGEN, Chatsworth CA), or an OLIGOTEX mRNA purification kit (QIAGEN). Alternatively, RNA was isolated directly from tissue lysates using other RNA isolation kits, e.g., the POLY(A)PURE mRNA purification kit (Ambion, Austin TX).

In some cases, Stratagene was provided with RNA and constructed the corresponding cDNA libraries. Otherwise, cDNA was synthesized and cDNA libraries were constructed with the UNIZAP vector system (Stratagene) or SUPERScript plasmid system (Life Technologies), using the recommended procedures or similar methods known in the art. (See, e.g., Ausubel, 1997, supra, units 5.1-6.6.) Reverse transcription was initiated using oligo d(T) or random primers. Synthetic oligonucleotide adapters were ligated to double stranded cDNA, and the cDNA was digested with the appropriate restriction enzyme or enzymes. For most libraries, the cDNA was size-selected (300-1000 bp) using SEPHACRYL S1000, SEPHAROSE CL2B, or SEPHAROSE CL4B column chromatography (Amersham Pharmacia Biotech) or preparative agarose gel electrophoresis. cDNAs were ligated into compatible restriction enzyme sites of the polylinker of a suitable plasmid, e.g.,

PBLUESCRIPT plasmid (Stratagene), PSPO1 plasmid (Life Technologies), pcDNA2.1 plasmid (Invitrogen, Carlsbad CA), or pINCY plasmid (Incyte Genomics, Palo Alto CA). Recombinant plasmids were transformed into competent *E. coli* cells including XL1-Blue, XL1-BlueMRF, or SOLR from Stratagene or DH5 α , DH10B, or ElectroMAX DH10B from Life Technologies.

5 II. Isolation of cDNA Clones

Plasmids obtained as described in Example I were recovered from host cells by *in vivo* excision using the UNIZAP vector system (Stratagene) or by cell lysis. Plasmids were purified using at least one of the following: a Magic or WIZARD Minipreps DNA purification system (Promega); an AGTC Miniprep purification kit (Edge Biosystems, Gaithersburg MD); and QIAWELL 8 Plasmid, QIAWELL 10 8 Plus Plasmid, QIAWELL 8 Ultra Plasmid purification systems or the R.E.A.L. PREP 96 plasmid purification kit from QIAGEN. Following precipitation, plasmids were resuspended in 0.1 ml of distilled water and stored, with or without lyophilization, at 4°C.

Alternatively, plasmid DNA was amplified from host cell lysates using direct link PCR in a high-throughput format (Rao, V.B. (1994) Anal. Biochem. 216:1-14). Host cell lysis and thermal 15 cycling steps were carried out in a single reaction mixture. Samples were processed and stored in 384-well plates, and the concentration of amplified plasmid DNA was quantified fluorometrically using PICOGREEN dye (Molecular Probes, Eugene OR) and a FLUOROSKAN II fluorescence scanner (Labsystems Oy, Helsinki, Finland).

20 III. Sequencing and Analysis

Incyte cDNA recovered in plasmids as described in Example II were sequenced as follows. Sequencing reactions were processed using standard methods or high-throughput instrumentation such as the ABI CATALYST 800 (PE Biosystems) thermal cycler or the PTC-200 thermal cycler (MJ Research) in conjunction with the HYDRA microdispenser (Robbins Scientific) or the MICROLAB 2200 (Hamilton) liquid transfer system. cDNA sequencing reactions were prepared using reagents 25 provided by Amersham Pharmacia Biotech or supplied in ABI sequencing kits such as the ABI PRISM BIGDYE Terminator cycle sequencing ready reaction kit (PE Biosystems). Electrophoretic separation of cDNA sequencing reactions and detection of labeled polynucleotides were carried out using the MEGABACE 1000 DNA sequencing system (Molecular Dynamics); the ABI PRISM 373 or 377 sequencing system (PE Biosystems) in conjunction with standard ABI protocols and base calling 30 software; or other sequence analysis systems known in the art. Reading frames within the cDNA sequences were identified using standard methods (reviewed in Ausubel, 1997, *supra*, unit 7.7). Some of the cDNA sequences were selected for extension using the techniques disclosed in Example VI.

The polynucleotide sequences derived from cDNA sequencing were assembled and analyzed using a combination of software programs which utilize algorithms well known to those skilled in the

art. Table 5 summarizes the tools, programs, and algorithms used and provides applicable descriptions, references, and threshold parameters. The first column of Table 5 shows the tools, programs, and algorithms used, the second column provides brief descriptions thereof, the third column presents appropriate references, all of which are incorporated by reference herein in their entirety, and the fourth column presents, where applicable, the scores, probability values, and other parameters used to evaluate the strength of a match between two sequences (the higher the score, the greater the homology between two sequences). Sequences were analyzed using MACDNASIS PRO software (Hitachi Software Engineering, South San Francisco CA) and LASERGENE software (DNASTAR). Polynucleotide and polypeptide sequence alignments were generated using the default parameters specified by the clustal algorithm as incorporated into the MEGALIGN multisequence alignment program (DNASTAR), which also calculates the percent identity between aligned sequences.

The polynucleotide sequences were validated by removing vector, linker, and polyA sequences and by masking ambiguous bases, using algorithms and programs based on BLAST, dynamic programming, and dinucleotide nearest neighbor analysis. The sequences were then queried against a selection of public databases such as the GenBank primate, rodent, mammalian, vertebrate, and eukaryote databases, and BLOCKS, PRINTS, DOMO, PRODOM, and PFAM to acquire annotation using programs based on BLAST, FASTA, and BLIMPS. The sequences were assembled into full length polynucleotide sequences using programs based on Phred, Phrap, and Consed, and were screened for open reading frames using programs based on GeneMark, BLAST, and FASTA. The full length polynucleotide sequences were translated to derive the corresponding full length amino acid sequences, and these full length sequences were subsequently analyzed by querying against databases such as the GenBank databases (described above), SwissProt, BLOCKS, PRINTS, DOMO, PRODOM, Prosite, and Hidden Markov Model (HMM)-based protein family databases such as PFAM. HMM is a probabilistic approach which analyzes consensus primary structures of gene families. (See, e.g., Eddy, S.R. (1996) *Curr. Opin. Struct. Biol.* 6:361-365.)

The programs described above for the assembly and analysis of full length polynucleotide and amino acid sequences were also used to identify polynucleotide sequence fragments from SEQ ID NO:15-28. Fragments from about 20 to about 4000 nucleotides which are useful in hybridization and amplification technologies were described in The Invention section above.

IV. Analysis of Polynucleotide Expression

Northern analysis is a laboratory technique used to detect the presence of a transcript of a gene and involves the hybridization of a labeled nucleotide sequence to a membrane on which RNAs from a particular cell type or tissue have been bound. (See, e.g., Sambrook, *supra*, ch. 7; Ausubel, 1995, *supra*, ch. 4 and 16.)

Analogous computer techniques applying BLAST were used to search for identical or related molecules in cDNA databases such as GenBank or LIFESEQ (Incyte Genomics). This analysis is much faster than multiple membrane-based hybridizations. In addition, the sensitivity of the computer search can be modified to determine whether any particular match is categorized as exact or similar.

- 5 The basis of the search is the product score, which is defined as:

$$\frac{\text{BLAST Score} \times \text{Percent Identity}}{5 \times \text{minimum} \{ \text{length}(\text{Seq. 1}), \text{length}(\text{Seq. 2}) \}}$$

- 10 The product score takes into account both the degree of similarity between two sequences and the length of the sequence match. The product score is a normalized value between 0 and 100, and is calculated as follows: the BLAST score is multiplied by the percent nucleotide identity and the product is divided by (5 times the length of the shorter of the two sequences). The BLAST score is calculated by assigning a score of +5 for every base that matches in a high-scoring segment pair (HSP), and -4 for every mismatch. Two sequences may share more than one HSP (separated by gaps). If there is more
15 than one HSP, then the pair with the highest BLAST score is used to calculate the product score. The product score represents a balance between fractional overlap and quality in a BLAST alignment. For example, a product score of 100 is produced only for 100% identity over the entire length of the shorter of the two sequences being compared. A product score of 70 is produced either by 100% identity and 70% overlap at one end, or by 88% identity and 100% overlap at the other. A product score of 50 is
20 produced either by 100% identity and 50% overlap at one end, or 79% identity and 100% overlap.

- The results of northern analyses are reported as a percentage distribution of libraries in which the transcript encoding HYENZ occurred. Analysis involved the categorization of cDNA libraries by organ/tissue and disease. The organ/tissue categories included cardiovascular, dermatologic, developmental, endocrine, gastrointestinal, hematopoietic/immune, musculoskeletal, nervous,
25 reproductive, and urologic. The disease/condition categories included cancer, inflammation, trauma, cell proliferation, neurological, and pooled. For each category, the number of libraries expressing the sequence of interest was counted and divided by the total number of libraries across all categories. Percentage values of tissue-specific and disease- or condition-specific expression are reported in Table 3.

30 **V. Chromosomal Mapping of HYENZ Encoding Polynucleotides**

The cDNA sequences which were used to assemble SEQ ID NO:15-28 were compared with sequences from the Incyte LIFESEQ database and public domain databases using BLAST and other implementations of the Smith-Waterman algorithm. Sequences from these databases that matched SEQ ID NO:15-28 were assembled into clusters of contiguous and overlapping sequences using

assembly algorithms such as Phrap (Table 5). Radiation hybrid and genetic mapping data available from public resources such as the Stanford Human Genome Center (SHGC), Whitehead Institute for Genome Research (WIGR), and Généthon were used to determine if any of the clustered sequences had been previously mapped. Inclusion of a mapped sequence in a cluster resulted in the assignment of all sequences of that cluster, including its particular SEQ ID NO., to that map location.

The genetic map location of SEQ ID NO: 19 is described in The Invention as a range, or interval, of a human chromosome. The map position of an interval, in centiMorgans, is measured relative to the terminus of the chromosome's p-arm. (The centiMorgan (cM) is a unit of measurement based on recombination frequencies between chromosomal markers. On average, 1 cM is roughly equivalent to 1 megabase (Mb) of DNA in humans, although this can vary widely due to hot and cold spots of recombination.) The cM distances are based on genetic markers mapped by Généthon which provide boundaries for radiation hybrid markers whose sequences were included in each of the clusters. Human genome maps and other resources available to the public, such as the NCBI "GeneMap'99" World Wide Web site (<http://www.ncbi.nlm.nih.gov/genemap/>), can be employed to determine if previously identified disease genes map within or in proximity to the intervals indicated above.

VI. Extension of HYENZ Encoding Polynucleotides

The full length nucleic acid sequences of SEQ ID NO:15-28 were produced by extension of an appropriate fragment of the full length molecule using oligonucleotide primers designed from this fragment. One primer was synthesized to initiate 5' extension of the known fragment, and the other primer, to initiate 3' extension of the known fragment. The initial primers were designed using OLIGO 4.06 software (National Biosciences), or another appropriate program, to be about 22 to 30 nucleotides in length, to have a GC content of about 50% or more, and to anneal to the target sequence at temperatures of about 68°C to about 72°C. Any stretch of nucleotides which would result in hairpin structures and primer-primer dimerizations was avoided.

Selected human cDNA libraries were used to extend the sequence. If more than one extension was necessary or desired, additional or nested sets of primers were designed.

High fidelity amplification was obtained by PCR using methods well known in the art. PCR was performed in 96-well plates using the PTC-200 thermal cycler (MJ Research, Inc.). The reaction mix contained DNA template, 200 nmol of each primer, reaction buffer containing Mg^{2+} , $(NH_4)_2SO_4$, and β -mercaptoethanol, Taq DNA polymerase (Amersham Pharmacia Biotech), ELONGASE enzyme (Life Technologies), and Pfu DNA polymerase (Stratagene), with the following parameters for primer pair PCI A and PCI B: Step 1: 94°C, 3 min; Step 2: 94°C, 15 sec; Step 3: 60°C, 1 min; Step 4: 68°C, 2 min; Step 5: Steps 2, 3, and 4 repeated 20 times; Step 6: 68°C, 5 min; Step 7: storage at 4°C. In the alternative, the parameters for primer pair T7 and SK+ were as follows: Step 1: 94°C, 3 min; Step 2:

94°C, 15 sec; Step 3: 57°C, 1 min; Step 4: 68°C, 2 min; Step 5: Steps 2, 3, and 4 repeated 20 times; Step 6: 68°C, 5 min; Step 7: storage at 4°C.

The concentration of DNA in each well was determined by dispensing 100 µl PICOGREEN quantitation reagent (0.25% (v/v) PICOGREEN; Molecular Probes, Eugene OR) dissolved in 1X TE and 0.5 µl of undiluted PCR product into each well of an opaque fluorimeter plate (Corning Costar, Acton MA), allowing the DNA to bind to the reagent. The plate was scanned in a Fluoroskan II (Labsystems Oy, Helsinki, Finland) to measure the fluorescence of the sample and to quantify the concentration of DNA. A 5 µl to 10 µl aliquot of the reaction mixture was analyzed by electrophoresis on a 1 % agarose mini-gel to determine which reactions were successful in extending the sequence.

The extended nucleotides were desalted and concentrated, transferred to 384-well plates, digested with CviJI cholera virus endonuclease (Molecular Biology Research, Madison WI), and sonicated or sheared prior to religation into pUC 18 vector (Amersham Pharmacia Biotech). For shotgun sequencing, the digested nucleotides were separated on low concentration (0.6 to 0.8%) agarose gels, fragments were excised, and agar digested with Agar ACE (Promega). Extended clones were religated using T4 ligase (New England Biolabs, Beverly MA) into pUC 18 vector (Amersham Pharmacia Biotech), treated with Pfu DNA polymerase (Stratagene) to fill-in restriction site overhangs, and transfected into competent *E. coli* cells. Transformed cells were selected on antibiotic-containing media, and individual colonies were picked and cultured overnight at 37°C in 384-well plates in LB/2x carb liquid media.

The cells were lysed, and DNA was amplified by PCR using Taq DNA polymerase (Amersham Pharmacia Biotech) and Pfu DNA polymerase (Stratagene) with the following parameters: Step 1: 94°C, 3 min; Step 2: 94°C, 15 sec; Step 3: 60°C, 1 min; Step 4: 72°C, 2 min; Step 5: steps 2, 3, and 4 repeated 29 times; Step 6: 72°C, 5 min; Step 7: storage at 4°C. DNA was quantified by PICOGREEN reagent (Molecular Probes) as described above. Samples with low DNA recoveries were reamplified using the same conditions as described above. Samples were diluted with 20% dimethylsulfoxide (1:2, v/v), and sequenced using DYENAMIC energy transfer sequencing primers and the DYENAMIC DIRECT kit (Amersham Pharmacia Biotech) or the ABI PRISM BIGDYE Terminator cycle sequencing ready reaction kit (PE Biosystems).

In like manner, the polynucleotide sequences of SEQ ID NO:15-28 are used to obtain 5' regulatory sequences using the procedure above, along with oligonucleotides designed for such extension, and an appropriate genomic library.

VII. Labeling and Use of Individual Hybridization Probes

Hybridization probes derived from SEQ ID NO:15-28 are employed to screen cDNAs, genomic DNAs, or mRNAs. Although the labeling of oligonucleotides, consisting of about 20 base pairs, is

specifically described, essentially the same procedure is used with larger nucleotide fragments.

Oligonucleotides are designed using state-of-the-art software such as OLIGO 4.06 software (National Biosciences) and labeled by combining 50 pmol of each oligomer, 250 μ Ci of [γ - 32 P] adenosine triphosphate (Amersham Pharmacia Biotech), and T4 polynucleotide kinase (DuPont NEN, Boston MA). The labeled oligonucleotides are substantially purified using a SEPHADEX G-25 superfine size exclusion dextran bead column (Amersham Pharmacia Biotech). An aliquot containing 10^7 counts per minute of the labeled probe is used in a typical membrane-based hybridization analysis of human genomic DNA digested with one of the following endonucleases: Ase I, Bgl II, Eco RI, Pst I, Xba I, or Pvu II (DuPont NEN).

10 The DNA from each digest is fractionated on a 0.7% agarose gel and transferred to nylon membranes (Nytran Plus, Schleicher & Schuell, Durham NH). Hybridization is carried out for 16 hours at 40°C. To remove nonspecific signals, blots are sequentially washed at room temperature under conditions of up to, for example, 0.1 x saline sodium citrate and 0.5% sodium dodecyl sulfate. Hybridization patterns are visualized using autoradiography or an alternative imaging means and
15 compared.

VIII. Microarrays

The linkage or synthesis of array elements upon a microarray can be achieved utilizing photolithography, piezoelectric printing (ink-jet printing, See, e.g., Baldeschweiler, supra), mechanical microspotting technologies, and derivatives thereof. The substrate in each of the aforementioned
20 technologies should be uniform and solid with a non-porous surface (Schena (1999), supra). Suggested substrates include silicon, silica, glass slides, glass chips, and silicon wafers. Alternatively, a procedure analogous to a dot or slot blot may also be used to arrange and link elements to the surface of a substrate using thermal, UV, chemical, or mechanical bonding procedures. A typical array may be produced using available methods and machines well known to those of ordinary skill in the art and may
25 contain any appropriate number of elements. (See, e.g., Schena, M. et al. (1995) Science 270:467-470; Shalon, D. et al. (1996) Genome Res. 6:639-645; Marshall, A. and J. Hodgson (1998) Nat. Biotechnol. 16:27-31.)

Full length cDNAs, Expressed Sequence Tags (ESTs), or fragments or oligomers thereof may comprise the elements of the microarray. Fragments or oligomers suitable for hybridization can be
30 selected using software well known in the art such as LASERGENE software (DNASTAR). The array elements are hybridized with polynucleotides in a biological sample. The polynucleotides in the biological sample are conjugated to a fluorescent label or other molecular tag for ease of detection. After hybridization, nonhybridized nucleotides from the biological sample are removed, and a fluorescence scanner is used to detect hybridization at each array element. Alternatively, laser

desorption and mass spectrometry may be used for detection of hybridization. The degree of complementarity and the relative abundance of each polynucleotide which hybridizes to an element on the microarray may be assessed. In one embodiment, microarray preparation and usage is described in detail below.

5 Tissue or Cell Sample Preparation

Total RNA is isolated from tissue samples using the guanidinium thiocyanate method and poly(A)⁺ RNA is purified using the oligo-(dT) cellulose method. Each poly(A)⁺ RNA sample is reverse transcribed using MMLV reverse-transcriptase, 0.05 pg/ μ l oligo-(dT) primer (21mer), 1X first strand buffer, 0.03 units/ μ l RNase inhibitor, 500 μ M dATP, 500 μ M dGTP, 500 μ M dTTP, 40 μ M dCTP, 40 μ M dCTP-Cy3 (BDS) or dCTP-Cy5 (Amersham Pharmacia Biotech). The reverse transcription reaction is performed in a 25 ml volume containing 200 ng poly(A)⁺ RNA with GEMBRIGHT kits (Incyte). Specific control poly(A)⁺ RNAs are synthesized by in vitro transcription from non-coding yeast genomic DNA. After incubation at 37°C for 2 hr, each reaction sample (one with Cy3 and another with Cy5 labeling) is treated with 2.5 ml of 0.5M sodium hydroxide and incubated for 20 minutes at 85°C to stop the reaction and degrade the RNA. Samples are purified using two successive CHROMA SPIN 30 gel filtration spin columns (CLONTECH Laboratories, Inc. (CLONTECH), Palo Alto CA) and after combining, both reaction samples are ethanol precipitated using 1 ml of glycogen (1 mg/ml), 60 ml sodium acetate, and 300 ml of 100% ethanol. The sample is then dried to completion using a SpeedVAC (Savant Instruments Inc., Holbrook NY) and resuspended in 14 μ l 5X SSC/0.2% SDS.

20 Microarray Preparation

Sequences of the present invention are used to generate array elements. Each array element is amplified from bacterial cells containing vectors with cloned cDNA inserts. PCR amplification uses primers complementary to the vector sequences flanking the cDNA insert. Array elements are amplified in thirty cycles of PCR from an initial quantity of 1-2 ng to a final quantity greater than 5 μ g. Amplified array elements are then purified using SEPHACRYL-400 (Amersham Pharmacia Biotech).

Purified array elements are immobilized on polymer-coated glass slides. Glass microscope slides (Corning) are cleaned by ultrasound in 0.1% SDS and acetone, with extensive distilled water washes between and after treatments. Glass slides are etched in 4% hydrofluoric acid (VWR Scientific Products Corporation (VWR), West Chester PA), washed extensively in distilled water, and coated with 0.05% aminopropyl silane (Sigma) in 95% ethanol. Coated slides are cured in a 110°C oven.

Array elements are applied to the coated glass substrate using a procedure described in US Patent No. 5,807,522, incorporated herein by reference. 1 μ l of the array element DNA, at an average

concentration of 100 ng/ μ l, is loaded into the open capillary printing element by a high-speed robotic apparatus. The apparatus then deposits about 5 nl of array element sample per slide.

Microarrays are UV-crosslinked using a STRATALINKER UV-crosslinker (Stratagene). Microarrays are washed at room temperature once in 0.2% SDS and three times in distilled water.

- 5 Non-specific binding sites are blocked by incubation of microarrays in 0.2% casein in phosphate buffered saline (PBS) (Tropix, Inc., Bedford MA) for 30 minutes at 60 °C followed by washes in 0.2% SDS and distilled water as before.

Hybridization

- Hybridization reactions contain 9 μ l of sample mixture consisting of 0.2 μ g each of Cy3 and
10 Cy5 labeled cDNA synthesis products in 5X SSC, 0.2% SDS hybridization buffer. The sample mixture is heated to 65 °C for 5 minutes and is aliquoted onto the microarray surface and covered with an 1.8 cm² coverslip. The arrays are transferred to a waterproof chamber having a cavity just slightly larger than a microscope slide. The chamber is kept at 100% humidity internally by the addition of 140 μ l of 5X SSC in a corner of the chamber. The chamber containing the arrays is incubated for
15 about 6.5 hours at 60 °C. The arrays are washed for 10 min at 45 °C in a first wash buffer (1X SSC, 0.1% SDS), three times for 10 minutes each at 45 °C in a second wash buffer (0.1X SSC), and dried.

Detection

- Reporter-labeled hybridization complexes are detected with a microscope equipped with an Innova 70 mixed gas 10 W laser (Coherent, Inc., Santa Clara CA) capable of generating spectral lines
20 at 488 nm for excitation of Cy3 and at 632 nm for excitation of Cy5. The excitation laser light is focused on the array using a 20X microscope objective (Nikon, Inc., Melville NY). The slide containing the array is placed on a computer-controlled X-Y stage on the microscope and raster-scanned past the objective. The 1.8 cm x 1.8 cm array used in the present example is scanned with a resolution of 20 micrometers.

- 25 In two separate scans, a mixed gas multiline laser excites the two fluorophores sequentially. Emitted light is split, based on wavelength, into two photomultiplier tube detectors (PMT R1477, Hamamatsu Photonics Systems, Bridgewater NJ) corresponding to the two fluorophores. Appropriate filters positioned between the array and the photomultiplier tubes are used to filter the signals. The emission maxima of the fluorophores used are 565 nm for Cy3 and 650 nm for Cy5. Each array is
30 typically scanned twice, one scan per fluorophore using the appropriate filters at the laser source, although the apparatus is capable of recording the spectra from both fluorophores simultaneously.

- The sensitivity of the scans is typically calibrated using the signal intensity generated by a cDNA control species added to the sample mixture at a known concentration. A specific location on the array contains a complementary DNA sequence, allowing the intensity of the signal at that
35 location to be correlated with a weight ratio of hybridizing species of 1:100,000. When two samples

from different sources (e.g., representing test and control cells), each labeled with a different fluorophore, are hybridized to a single array for the purpose of identifying genes that are differentially expressed, the calibration is done by labeling samples of the calibrating cDNA with the two fluorophores and adding identical amounts of each to the hybridization mixture.

5 The output of the photomultiplier tube is digitized using a 12-bit RTI-835H analog-to-digital (A/D) conversion board (Analog Devices, Inc., Norwood MA) installed in an IBM-compatible PC computer. The digitized data are displayed as an image where the signal intensity is mapped using a linear 20-color transformation to a pseudocolor scale ranging from blue (low signal) to red (high signal). The data is also analyzed quantitatively. Where two different fluorophores are excited and
10 measured simultaneously, the data are first corrected for optical crosstalk (due to overlapping emission spectra) between the fluorophores using each fluorophore's emission spectrum.

A grid is superimposed over the fluorescence signal image such that the signal from each spot is centered in each element of the grid. The fluorescence signal within each element is then integrated to obtain a numerical value corresponding to the average intensity of the signal. The software used
15 for signal analysis is the GEMTOOLS gene expression analysis program (Incyte).

IX. Complementary Polynucleotides

Sequences complementary to the HYENZ-encoding sequences, or any parts thereof, are used to detect, decrease, or inhibit expression of naturally occurring HYENZ. Although use of oligonucleotides comprising from about 15 to 30 base pairs is described, essentially the same procedure is used with
20 smaller or with larger sequence fragments. Appropriate oligonucleotides are designed using OLIGO 4.06 software (National Biosciences) and the coding sequence of HYENZ. To inhibit transcription, a complementary oligonucleotide is designed from the most unique 5' sequence and used to prevent promoter binding to the coding sequence. To inhibit translation, a complementary oligonucleotide is designed to prevent ribosomal binding to the HYENZ-encoding transcript.

X. Expression of HYENZ

25 Expression and purification of HYENZ is achieved using bacterial or virus-based expression systems. For expression of HYENZ in bacteria, cDNA is subcloned into an appropriate vector containing an antibiotic resistance gene and an inducible promoter that directs high levels of cDNA transcription. Examples of such promoters include, but are not limited to, the *trp-lac (tac)* hybrid
30 promoter and the T5 or T7 bacteriophage promoter in conjunction with the *lac* operator regulatory element. Recombinant vectors are transformed into suitable bacterial hosts, e.g., BL21(DE3). Antibiotic resistant bacteria express HYENZ upon induction with isopropyl beta-D-thiogalactopyranoside (IPTG). Expression of HYENZ in eukaryotic cells is achieved by infecting insect or mammalian cell lines with recombinant Autographica californica nuclear polyhedrosis virus
35 (AcMNPV), commonly known as baculovirus. The nonessential polyhedrin gene of baculovirus is

replaced with cDNA encoding HYENZ by either homologous recombination or bacterial-mediated transposition involving transfer plasmid intermediates. Viral infectivity is maintained and the strong polyhedrin promoter drives high levels of cDNA transcription. Recombinant baculovirus is used to infect Spodoptera frugiperda (Sf9) insect cells in most cases, or human hepatocytes, in some cases.

- 5 Infection of the latter requires additional genetic modifications to baculovirus. (See Engelhard, E.K. et al. (1994) Proc. Natl. Acad. Sci. USA 91:3224-3227; Sandig, V. et al. (1996) Hum. Gene Ther. 7:1937-1945.)

In most expression systems, HYENZ is synthesized as a fusion protein with, e.g., glutathione S-transferase (GST) or a peptide epitope tag, such as FLAG or 6-His, permitting rapid, single-step, affinity-based purification of recombinant fusion protein from crude cell lysates. GST, a 26-kilodalton enzyme from Schistosoma japonicum, enables the purification of fusion proteins on immobilized glutathione under conditions that maintain protein activity and antigenicity (Amersham Pharmacia Biotech). Following purification, the GST moiety can be proteolytically cleaved from HYENZ at specifically engineered sites. FLAG, an 8-amino acid peptide, enables immunoaffinity purification using commercially available monoclonal and polyclonal anti-FLAG antibodies (Eastman Kodak). 6-His, a stretch of six consecutive histidine residues, enables purification on metal-chelate resins (QIAGEN). Methods for protein expression and purification are discussed in Ausubel (1995, supra, ch. 10 and 16). Purified HYENZ obtained by these methods can be used directly in the assays shown in Examples XI and XV.

20 **XI. Demonstration of HYENZ Activity**

For purposes of example, an assay measuring the β -glucosidase activity of an HYENZ molecule is described. Varying amounts of HYENZ are incubated with 1 mM 4-nitrophenyl β -D-glycopyranoside (a substrate) in 50 mM sodium acetate buffer, pH 5.0, for various times (typically 1-5 minutes) at 37°C. The reaction is halted by heating to 100°C for 2 minutes. The absorbance is measured spectrophotometrically at 410 nm, and is proportional to the activity of HYENZ in the sample. (Hrmova, M. et al. (1998) J. Biol. Chem. 273:11134-11143.)

XII. Functional Assays

HYENZ function is assessed by expressing the sequences encoding HYENZ at physiologically elevated levels in mammalian cell culture systems. cDNA is subcloned into a mammalian expression vector containing a strong promoter that drives high levels of cDNA expression. Vectors of choice include pCMV SPORT plasmid (Life Technologies) and pCR3.1 plasmid (Invitrogen), both of which contain the cytomegalovirus promoter. 5-10 μ g of recombinant vector are transiently transfected into a human cell line, for example, an endothelial or hematopoietic cell line, using either liposome formulations or electroporation. 1-2 μ g of an additional plasmid containing sequences encoding a

marker protein are co-transfected. Expression of a marker protein provides a means to distinguish transfected cells from nontransfected cells and is a reliable predictor of cDNA expression from the recombinant vector. Marker proteins of choice include, e.g., Green Fluorescent Protein (GFP; Clontech), CD64, or a CD64-GFP fusion protein. Flow cytometry (FCM), an automated, laser optics-based technique, is used to identify transfected cells expressing GFP or CD64-GFP and to evaluate the apoptotic state of the cells and other cellular properties. FCM detects and quantifies the uptake of fluorescent molecules that diagnose events preceding or coincident with cell death. These events include changes in nuclear DNA content as measured by staining of DNA with propidium iodide; changes in cell size and granularity as measured by forward light scatter and 90 degree side light scatter; down-regulation of DNA synthesis as measured by decrease in bromodeoxyuridine uptake; alterations in expression of cell surface and intracellular proteins as measured by reactivity with specific antibodies; and alterations in plasma membrane composition as measured by the binding of fluorescein-conjugated Annexin V protein to the cell surface. Methods in flow cytometry are discussed in Ormerod, M.G. (1994) Flow Cytometry, Oxford, New York NY.

The influence of HYENZ on gene expression can be assessed using highly purified populations of cells transfected with sequences encoding HYENZ and either CD64 or CD64-GFP. CD64 and CD64-GFP are expressed on the surface of transfected cells and bind to conserved regions of human immunoglobulin G (IgG). Transfected cells are efficiently separated from nontransfected cells using magnetic beads coated with either human IgG or antibody against CD64 (DYNAL, Lake Success NY). mRNA can be purified from the cells using methods well known by those of skill in the art. Expression of mRNA encoding HYENZ and other genes of interest can be analyzed by northern analysis or microarray techniques.

XIII. Production of HYENZ Specific Antibodies

HYENZ substantially purified using polyacrylamide gel electrophoresis (PAGE; see, e.g., Harrington, M.G. (1990) *Methods Enzymol.* 182:488-495), or other purification techniques, is used to immunize rabbits and to produce antibodies using standard protocols.

Alternatively, the HYENZ amino acid sequence is analyzed using LASERGENE software (DNASTAR) to determine regions of high immunogenicity, and a corresponding oligopeptide is synthesized and used to raise antibodies by means known to those of skill in the art. Methods for selection of appropriate epitopes, such as those near the C-terminus or in hydrophilic regions are well described in the art. (See, e.g., Ausubel, 1995, *supra*, ch. 11.)

Typically, oligopeptides of about 15 residues in length are synthesized using an ABI 431A peptide synthesizer (PE Biosystems) using FMOC chemistry and coupled to KLH (Sigma-Aldrich, St. Louis MO) by reaction with N-maleimidobenzoyl-N-hydroxysuccinimide ester (MBS) to increase

immunogenicity. (See, e.g., Ausubel, 1995, supra.) Rabbits are immunized with the oligopeptide-KLH complex in complete Freund's adjuvant. Resulting antisera are tested for antipeptide and anti-HYENZ activity by, for example, binding the peptide or HYENZ to a substrate, blocking with 1% BSA, reacting with rabbit antisera, washing, and reacting with radio-iodinated goat anti-rabbit IgG.

5 **XIV. Purification of Naturally Occurring HYENZ Using Specific Antibodies**

Naturally occurring or recombinant HYENZ is substantially purified by immunoaffinity chromatography using antibodies specific for HYENZ. An immunoaffinity column is constructed by covalently coupling anti-HYENZ antibody to an activated chromatographic resin, such as CNBr-activated SEPHAROSE (Amersham Pharmacia Biotech). After the coupling, the resin is
10 blocked and washed according to the manufacturer's instructions.

Media containing HYENZ are passed over the immunoaffinity column, and the column is washed under conditions that allow the preferential absorbance of HYENZ (e.g., high ionic strength buffers in the presence of detergent). The column is eluted under conditions that disrupt antibody/HYENZ binding (e.g., a buffer of pH 2 to pH 3, or a high concentration of a chaotrope, such
15 as urea or thiocyanate ion), and HYENZ is collected.

XV. Identification of Molecules Which Interact with HYENZ

HYENZ, or biologically active fragments thereof, are labeled with ¹²⁵I Bolton-Hunter reagent. (See, e.g., Bolton A.E. and W.M. Hunter (1973) Biochem. J. 133:529-539.) Candidate molecules previously arrayed in the wells of a multi-well plate are incubated with the labeled HYENZ, washed,
20 and any wells with labeled HYENZ complex are assayed. Data obtained using different concentrations of HYENZ are used to calculate values for the number, affinity, and association of HYENZ with the candidate molecules.

Alternatively, molecules interacting with HYENZ are analyzed using the yeast two-hybrid system as described in Fields, S. and O. Song (1989, Nature 340:245-246), or using commercially
25 available kits based on the two-hybrid system, such as the MATCHMAKER system (Clontech).

HYENZ may also be used in the PATHCALLING process (CuraGen Corp., New Haven CT) which employs the yeast two-hybrid system in a high-throughput manner to determine all interactions between the proteins encoded by two large libraries of genes (Nandabalan, K. et al. (2000) U.S. Patent No. 6,057,101).

30

Various modifications and variations of the described methods and systems of the invention will be apparent to those skilled in the art without departing from the scope and spirit of the invention. Although the invention has been described in connection with certain embodiments, it should be understood that the invention as claimed should not be unduly limited to such specific embodiments.

Indeed, various modifications of the described modes for carrying out the invention which are obvious to those skilled in molecular biology or related fields are intended to be within the scope of the following claims.

TABLE 1

Polypeptide SEQ ID NO:	Nucleotide SEQ ID NO:	Clone ID	Library	Fragments
1	15	1659002	URETTUT01	1419114T1 (KIDNNOT09), 1513348F6 (PANCUTUT01), 1659002H1 (URETTUT01), 2510396F6 (CONUTUT01), 5608212H1 (MONOTXS05)
2	16	1881009	LEUKNOT03	515242R1 (MMLR1DT01), 900867R1 (BRSTTUT03), 948697R1 (PANCNOT05), 1440708F1 (THYRN0T03), 1513974T1 (PANCUTUT01), 1881009F6 and 1881009H1 (LEUKNOT03), 3664878H1 (PANCNOT16), 4647111H1 (PROSTUT20)
3	17	2054065	BEPINOT01	222269R1 (PANCNOT01), 274662R6 (PANCIDT03), 882151R1 (THYRN0T02), 996561H1 (KIDNTUT01), 1868521F6 (SKINBIT01), 2054065H1, 2054065T6, 2054065X23R1, and 2054065X24R1 (BEPINOT01), 3208022H1 (PENCNOT03), 4624711H1 (FIBRTXT02)
4	18	2183367	SININOT01	2053428T6 (BEPINOT01), 2183367T6 (SININOT01), 2189606H1 (PROSNOT26), 2246242R6 (HIPONON02)
5	19	2458536	ENDANOT01	1806579T6 (SINTNOT13), 2050775F6 (LIVRFET02), 2458536H1 (ENDANOT01), 3769120H1 (BRSTNOT24), SBHA02033F1, SBHA01031F1, SBHA02361F1
6	20	2472979	THP1NOT03	1344324H1 (PROSNOT11), 1959519R6 (BRSTNOT04), 2296250R6 (BRSTNOT05), 2472979F6 and 2472979H1 (THP1NOT03), 2672695F6 (KIDNNOT19), 2929079H1 (TLYMN0T04), 3337871H1 (SPLNNOT10), 3521758H1 (LUNGNON03), 3688966H1 (HEAANOT01), 4310094H1 (BRAUNOT01), 5264492H2 (CONDUTUT02), 5286514H1 (LIVRTUS02)
7	21	2612754	UTRSTUT05	1376855F1 (LUNGNOT10), 1454080F1 (PENITUT01), 1617023F6 (BRAITUT12), 2481271F6 (SMCANOT01), 2612754H1 and 2612754X302T3 (UTRSTUT05), 3053767T6 (LNODNOT08), 3435070F6 (PENCNOT05), 4215323H1 (ADREN0T15)
8	22	2616646	GBLANOT01	938230H1 (CERVNOT01), 969237R6 (BRSTNOT05), 1283020F6 and 1283020T6 (COLNNOT16), 1442777F6 (THYRN0T03), 1967639R6 (BRSTNOT04), 2616646H1 (GBLANOT01), 3222187H1 (COLNNON03), 3478572F6 (OVARNOT11), SBWA04634V1, g884974
9	23	2625111	PROSTUT12	1214365R6 (BRSTTUT01), 1316376H1 (BLADTUT02), 2625111H1 and 2625111T6 (PROSTUT12), SBKA00669F1
10	24	2724525	LUNGTUT10	004103H1 (HMCINOT01), 798699R6 (OVARNOT03), 963092R2 (BRSTTUT03), 1890931F6 (BLADTUT07), 2724525F6 and 2724525H1 (LUNGTUT10), 2785502F6 (BRSTNOT13)
11	25	2824691	ADRETUT06	1492752R6 and 1492752T1 (PROSNON01), 2824691F6 and 2824691H1 (ADRETUT06), 3229650H1 (COTRNOT01), 5063468F6 (ARTFIDT01), 5212888F6 (ENDMUNT01)

TABLE 1 (cont.)

Polypeptide SEQ ID NO:	Nucleotide SEQ ID NO:	Clone ID	Library	Fragments
12	26	4722794	COLCTUT02	1522169F6 (BLADTUT04), 1699132F6 (BLADTUT05), 2656074H1 (THYMNOT04), 2903776F6 (DRGCNOT01), 3664053T6 (PANCNOT16), 3664085F6 (PANCNOT16), 5603737H1 (MONOTXN03), 5611754H1 (MONOTXS05)
13	27	5328267	DRGTN04	5328267H1 and 5328758F6 (DRGTN04)
14	28	5382277	COLNNOT38	265659H1 (HNT2AGT01), 1494983T1 (PROSNON01), 1974971T6 (PANCNOT02), 5283447F6 (TESTNON04), 5382277H1 (COLNNOT38)

TABLE 2

Protein SEQ ID NO:	Amino Acid Residues	Potential Phosphorylation Sites	Potential Glycosylation Sites	Signature Sequence	Homologous Sequence	Analytical Methods
1	288	T172 S183 T237 S276 T278 S12 T136 S215 Y196	N95	Microbodies C-terminal targeting signal: D286 – L293 Hydrolase protein hydroxyacetylglutathione glyoxalase II probable intergenic region isozyme multigene: I43 – N205	enzyme involved in lipogenesis and lipolysis (g2960101) [Mycobacterium tuberculosis]	BLAST - GenBank, BLAST - PRODOM, MOTIFS
2	432	S48 T79 S104 S109 S122 S332 S392 T400 S420 S143 T343	N131 N330	Signal Peptide: M1 – Y19 Protein hydrolase glycosidase alpha trehalase acid precursor alpha-trehalose glucosylhydrolase glycoprotein: M1 – L156	ATH1 (g1061284) [Saccharomyces cerevisiae]	BLAST - GenBank, BLAST - PRODOM MOTIFS, SPScan
3	737	S297 S668 S15 S27 T52 T85 S108 T146 T177 T247 S317 S324 T374 T419 T425 S504 S505 T593 S630 T646 T657 T677 S699 T29 T231 S340 S457 S668 S701	N175 N192 N406	Cell attachment sequence: R248 – D250	dipeptidyl peptidase III (g4519883) [Homo sapiens]	BLAST - GenBank, MOTIFS
4	108	T2 S24 S48		Polypeptide deformylase: G62 – M93		BLIMPS - PFAM, MOTIFS
5	510	T25 S26 S242 S276 S298 T357 T380 S172 T291 S374 S479 S495	N122 N302	Exonuclease : V61 – G69, L187 – L199, H438 – M451		MOTIFS, BLIMPS - PFAM
6	732	S242 S509 S97 T188 S304 S357 S385 S386 S398 T400 S508 T671 S18 S31 T101 S317 S498 S529 S616	N95 N474 N580 N613 N686 N717		serine-rich protein (g3873550) [Schizosaccharomyces pombe] P38IP (g5163089) [Homo sapiens]	BLAST – GenBank, MOTIFS

TABLE 2 (cont.)

Protein SEQ ID NO:	Amino Acid Residues	Potential Phosphorylation Sites	Potential Glycosylation Sites	Signature Sequence	Homologous Sequence	Analytical Methods
7	343	S299 S312 S294 S334	N165	Signal Peptide: M1 - R35 Aminoacyl-transfer RNA synthetases class II: Y34 - D53 Transmembrane Domain: W263 - V281	phosphatidic acid phosphohydrolase type-2c (g2911498) [Homo sapiens]	BLAST - GenBank, MOTIFS, HMMER, SPScan
8	717	S413 S216 S236 S261 S271 S310 T361 S393 S423 T548 T590 S624 S712 S7 S125 S629 S650 S662 S698		Signal Peptide: M1 - G29 Lipases serine active site: L 482 - G491 Transmembrane Domains: Y103 - C123, K141 - W163, N176 - I198	MDGL precursor (g217986) [Penicillium camemberti]	BLAST - GenBank, MOTIFS, HMMER; SPScan
9	236	T86 S208 T22 S40 T50 S53 T57 S128		Signal Peptide: M1 - G43 MutT domain: V79 - V94		BLIMPS - PRINTS, MOTIFS, SPScan
10	386	S2 S52 T62 S72 S114 T186 T242 S249 T279 T66 T204 S215 S226 S269 T286 T294	N60	Lipase serine active site: E129 - I180 Epoxide hydrolase signature: L344 - F366 Esterase/hydrolase epoxide: T62 - V163 Hydrolase; tropinesterase; hydroxy; dehydrogenase: S74 - T204	similar to Hydrolase; cDNA EST EMBL: T00652 comes from this gene; cDNA (g4008339)	BLAST - GenBank, BLAST - PRODOM, BLAST - DOMO, BLIMPS - PRINTS, MOTIFS, ProfileScan
11	522	S66 T93 S181 T275 S301 S429 S479 S501 S509 S4 S31 S154 T245 Y488	N80 N155 N273 N309 N329 N346	Polyprotein endonuclease protease pol reverse transcriptase hydrolase RNA-directed DNA polymerase: V63 - K187 Pol Polyprotein: G83 - D262	protease, reverse transcriptase, ribonuclease H, integrase (g4539021) [Drosophila buzzatii]	BLAST - GenBank, BLAST - PRODOM, BLAST - DOMO, MOTIFS

TABLE 2 (cont.)

Protein SEQ ID NO:	Amino Acid Residues	Potential Phosphorylation Sites	Potential Glycosylation Sites	Signature Sequence	Homologous Sequence	Analytical Methods
12	420	S222 S8 T26 S35 S118 S223 S383 T188 S348	N227	ATP/GTP-binding site (P-loop): A117 - S124 Hydrolase N4 precursor: I119 - F164, G244 -E276 Hydrolase N4 precursor protein signal/ 1-asparaginase: K66 - G270	asparaginase related protein (g7800880) [Neurospora crassa]	BLAST - GenBank, BLAST - PRODOM, BLAST - DOMO, BLIMPS - PRODOM, MOTIFS
13	186	S42 S167 T29 T88 Y142		Signal Peptide: M1 - A18 Lysozyme: K19 - C146 α -lactalbumin/lysozyme C signature: C95 - C113 I74 - C134	precursor protein ; P lysozyme (g49676) [Mus musculus domesticus]	BLIMPS - BLOCKS, BLIMPS - PRINTS BLAST - GenBank, BLAST - PRODOM, BLAST - DOMO, MOTIFS, SPScan, HMMER - PFAM, HMMER, ProfileScan
14	248	S221 S38 T139 S201	N89	Signal Peptide: M1 - G36 Isoamyl acetate-hydrolyzing esterase, EC 3.1.-.- hydrolase: W15 - E235	isoamyl acetate hydrolytic enzyme (g2073519) [Saccharomyces cerevisiae]	BLAST - GenBank, BLAST - PRODOM, MOTIFS, SPScan

TABLE 3

SEQ ID NO:	Fragment	Tissue Expression (Fraction of Total)	Disease or Condition (Fraction of Total)	Vector
15	1 - 47 852 - 911	Cardiovascular (0.238) Gastrointestinal (0.238) Reproductive (0.190)	Cancer (0.548) Inflammation (0.333) Cell Proliferation (0.238)	pINCY
16	596 - 655 974 - 1033	Reproductive (0.216) Gastrointestinal (0.198) Hematopoietic/Immune (0.171) Cardiovascular (0.153)	Cancer (0.477) Inflammation (0.369) Cell Proliferation (0.135)	pINCY
17	536 - 595	Reproductive (0.231) Nervous (0.179) Hematopoietic/Immune (0.167) Cardiovascular (0.154) Gastrointestinal (0.128)	Cancer (0.410) Inflammation (0.397) Cell Proliferation (0.244)	PSPORT1
18	110 - 169	Reproductive (0.375) Dermatologic (0.125) Endocrine (0.125) Gastrointestinal (0.125) Hematopoietic/Immune (0.125) Nervous (0.125)	Inflammation (0.375) Cancer (0.250) Cell Proliferation (0.125)	pINCY
19	433 - 492 940 - 999	Nervous (0.233) Reproductive (0.233) Developmental (0.116) Gastrointestinal (0.116)	Cancer (0.419) Inflammation (0.279) Cell Proliferation (0.279)	PBLUESCRIPT
20	541 - 600 1027 - 1086	Reproductive (0.250) Hematopoietic/Immune (0.233) Gastrointestinal (0.150) Cardiovascular (0.117) Nervous (0.100)	Inflammation (0.484) Cancer (0.383) Cell Proliferation (0.200)	pINCY
21	487 - 546 919 - 978	Nervous (0.284) Reproductive (0.230) Gastrointestinal (0.122)	Cancer (0.500) Inflammation (0.311) Cell Proliferation (0.095)	pINCY

TABLE 3 (Cont.)

SEQ ID NO.	Fragment	Tissue Expression (Fraction of Total)	Disease or Condition (Fraction of Total)	Vector
22	651 - 710	Reproductive (0.245) Gastrointestinal (0.170) Nervous (0.170) Hematopoietic/Immune (0.132)	Cancer (0.491) Inflammation (0.358) Cell Proliferation (0.226)	pINCY
23	109 - 168 325 - 384	Gastrointestinal (0.429) Reproductive (0.286) Urologic (0.143)	Cancer (0.500) Inflammation (0.286)	pINCY
24	271 - 330	Reproductive (0.312) Nervous (0.203) Gastrointestinal (0.125)	Cancer (0.562) Inflammation (0.203) Cell Proliferation (0.188)	pINCY
25	650 - 709 1298 - 1357	Nervous (0.364) Reproductive (0.182) Cardiovascular (0.182)	Inflammation (0.637) Cancer (0.273) Cell Proliferation (0.091)	pINCY
26	272 - 331	Reproductive (0.296) Hematopoietic/Immune (0.222) Cardiovascular (0.148) Nervous (0.111)	Cancer (0.556) Inflammation (0.333) Cell Proliferation (0.148)	pINCY
27	489 - 548	Nervous (1.000)	Inflammation (1.000)	pINCY
28	103 - 162	Reproductive (0.208) Nervous (0.195) Cardiovascular (0.169) Hematopoietic/Immune (0.156)	Cancer (0.416) Inflammation (0.351) Cell Proliferation (0.234)	pINCY

TABLE 4

Nucleotide SEQ ID NO:	Library	Library Description
15	URETTUT01	The library was constructed using RNA isolated from right ureter tumor tissue of a 69-year-old Caucasian male during ureterectomy and lymph node excision. Pathology indicated invasive grade 3 transitional cell carcinoma. Patient history included benign colon neoplasm, asthma, emphysema, acute duodenal ulcer, and hyperplasia of the prostate. Family history included atherosclerotic coronary artery disease, congestive heart failure, and malignant lung neoplasm.
16	LEUKNOT03	The library was constructed using RNA isolated from white blood cells of a 27-year-old female with blood type A+. The donor tested negative for cytomegalovirus (CMV).
17	BEPINOT01	The library was constructed using RNA isolated from a bronchial epithelium primary cell line derived from a 54-year-old Caucasian male.
18	SININOT01	The library was constructed using RNA isolated from ileum tissue obtained from the small intestine of a 4-year-old Caucasian female, who died from a closed head injury. Patient history included jaundice. Previous surgeries included a double hernia repair.
19	ENDANOT01	The library was constructed using RNA isolated from aortic endothelial cell tissue from an explanted heart removed from a male during a heart transplant.
20	THPINOT03	The library was constructed using RNA isolated from untreated THP-1 cells. THP-1 (ATCC TIB 202) is a human promonocyte line derived from the peripheral blood of a 1-year-old Caucasian male with acute monocytic leukemia (ref: Int. J. Cancer (1980) 26:171).
21	UTRSTUT05	The library was constructed using RNA isolated from uterine tumor tissue removed from a 41-year-old Caucasian female during a vaginal hysterectomy with dilation and curettage. Pathology indicated uterine leiomyoma. The endometrium was secretory and contained fragments of endometrial polyps. Benign endo- and ectocervical mucosa were identified in the endocervix. Patient history included a ventral hernia and a benign ovarian neoplasm.
22	GBLANOT01	The library was constructed using RNA isolated from diseased gallbladder tissue removed from a 53-year-old Caucasian female during a cholecystectomy. Pathology indicated mild chronic cholecystitis and cholelithiasis with approximately 150 mixed gallstones. Family history included benign hypertension.
23	PROSTUT12	The library was constructed using RNA isolated from prostate tumor tissue removed from a 65-year-old Caucasian male during a radical prostatectomy. Pathology indicated an adenocarcinoma (Gleason grade 2+2). Adenofibromatous hyperplasia was also present. The patient presented with elevated prostate specific antigen (PSA).
24	LUNGTUT10	The library was constructed using RNA isolated from lung tumor tissue removed from the left upper lobe of a 65-year-old Caucasian female during a segmental lung resection. Pathology indicated a metastatic grade 2 myxoid liposarcoma and a metastatic grade 4 liposarcoma. Patient history included soft tissue cancer, breast cancer, and secondary lung cancer.
25	ADRETUT06	The library was constructed using RNA isolated from adrenal tumor tissue removed from a 57-year-old Caucasian female during a unilateral right adrenalectomy. Pathology indicated pheochromocytoma, forming a nodular mass completely replacing the medulla of the adrenal gland. The surgical margins were negative for tumor.

TABLE 4 (cont.)

Nucleotide SEQ ID NO:	Library	Library Description
26	COLCTUT02	The library was constructed using RNA isolated from colon tumor tissue removed from the cecum of a 30-year-old Caucasian female during partial colectomy, open liver biopsy, incidental appendectomy, and permanent colostomy. Pathology indicated carcinoid tumor (grade 1 neuroendocrine carcinoma) arising in the terminal ileum, forming a mass in the right colon. Patient history included chronic sinus infections and endometriosis. Family history included hyperlipidemia, anxiety, upper lobe lung cancer, stomach cancer, liver cancer, and cirrhosis.
27	DRGTNON04	The normalized dorsal root ganglion library was constructed from 5.64 million independent clones from a dorsal root ganglion library. Starting RNA was made from thoracic dorsal root ganglion tissue from a 32-year-old Caucasian male, who died from acute pulmonary edema, acute bronchopneumonia, pleural and pericardial effusion, and lymphoma. The patient presented with pyrexia, fatigue, and GI bleeding. The patient received radiation therapy. Patient history included probable cytomegalovirus infection, liver congestion and steatosis, splenomegaly, hemorrhagic cystitis, thyroid hemorrhage, respiratory failure, pneumonia, natural killer cell lymphoma of the pharynx, and Bell's palsy. The library was normalized in one round using conditions adapted from Soares et al., PNAS (1994) 91:9228 and Bonaldo et al., Genome Research 6 (1996):791, except that a significantly longer (48-hours/round) reannealing hybridization was used.
28	COLNNOT38	The library was constructed using RNA isolated from colon tissue removed from a Caucasian male fetus, who died from Patau's syndrome (trisomy 13) at 20-weeks' gestation.

Table 5

Program	Description	Reference	Parameter Threshold
ABI FACTURA	A program that removes vector sequences and masks ambiguous bases in nucleic acid sequences.	PE Biosystems, Foster City, CA.	
ABI/PARACEL FDF	A Fast Data Finder useful in comparing and annotating amino acid or nucleic acid sequences.	PE Biosystems, Foster City, CA; Paracel Inc., Pasadena, CA.	Mismatch <50%
ABI AutoAssembler	A program that assembles nucleic acid sequences.	PE Biosystems, Foster City, CA.	
BLAST	A Basic Local Alignment Search Tool useful in sequence similarity search for amino acid and nucleic acid sequences. BLAST includes five functions: blastp, blastn, blastx, tblastn, and tblastx.	Altschul, S.F. et al. (1990) J. Mol. Biol. 215:403-410; Altschul, S.F. et al. (1997) Nucleic Acids Res. 25:3389-3402.	ESTs: Probability value= 1.0E-8 or less Full Length sequences: Probability value= 1.0E-10 or less
FASTA	A Pearson and Lipman algorithm that searches for similarity between a query sequence and a group of sequences of the same type. FASTA comprises at least five functions: fasta, tfasta, fastx, tfastx, and ssearch.	Pearson, W.R. and D.J. Lipman (1988) Proc. Natl. Acad. Sci. USA 85:2444-2448; Pearson, W.R. (1990) Methods Enzymol. 183:63-98; and Smith, T.F. and M.S. Waterman (1981) Adv. Appl. Math. 2:482-489.	ESTs: fasta E value=1.06E-6 Assembled ESTs: fasta Identity= 95% or greater and Match length=200 bases or greater; fastx E value=1.0E-8 or less Full Length sequences: fastx score=100 or greater
BLIMPS	A BLocks IMProved Searcher that matches a sequence against those in BLOCKS, PRINTS, DOMO, PRODOM, and PFAM databases to search for gene families, sequence homology, and structural fingerprint regions.	Henikoff, S. and J.G. Henikoff (1991) Nucleic Acids Res. 19:6565-6572; Henikoff, J.G. and S. Henikoff (1996) Methods Enzymol. 266:88-105; and Attwood, T.K. et al. (1997) J. Chem. Inf. Comput. Sci. 37:417-424.	Score=1000 or greater; Ratio of Score/Strength = 0.75 or larger; and, if applicable, Probability value= 1.0E-3 or less
HMMER	An algorithm for searching a query sequence against hidden Markov model (HMM)-based databases of protein family consensus sequences, such as PFAM.	Krogh, A. et al. (1994) J. Mol. Biol. 235:1501-1531; Sonnhammer, E.L.L. et al. (1988) Nucleic Acids Res. 26:320-322.	Score=10-50 bits for PFAM hits, depending on individual protein families

Table 5 (cont.)

Program	Description	Reference	Parameter Threshold
ProfilesScan	An algorithm that searches for structural and sequence motifs in protein sequences that match sequence patterns defined in Prosite.	Gribskov, M. et al. (1988) CABIOS 4:61-66; Gribskov, M. et al. (1989) Methods Enzymol. 183:146-159; Bairoch, A. et al. (1997) Nucleic Acids Res. 25:217-221.	Normalized quality score \geq GCG-specified "HIGH" value for that particular Prosite motif. Generally, score=1.4-2.1.
Phred	A base-calling algorithm that examines automated sequencer traces with high sensitivity and probability.	Ewing, B. et al. (1998) Genome Res. 8:175-185; Ewing, B. and P. Green (1998) Genome Res. 8:186-194.	
Phrap	A Phils Revised Assembly Program including SWAT and CrossMatch, programs based on efficient implementation of the Smith-Waterman algorithm, useful in searching sequence homology and assembling DNA sequences.	Smith, T.F. and M.S. Waterman (1981) Adv. Appl. Math. 2:482-489; Smith, T.F. and M.S. Waterman (1981) J. Mol. Biol. 147:195-197; and Green, P., University of Washington, Seattle, WA.	Score= 120 or greater; Match length= 56 or greater
Consed	A graphical tool for viewing and editing Phrap assemblies.	Gordon, D. et al. (1998) Genome Res. 8:195-202.	
SPScan	A weight matrix analysis program that scans protein sequences for the presence of secretory signal peptides.	Nielson, H. et al. (1997) Protein Engineering 10:1-6; Claverie, J.M. and S. Audic (1997) CABIOS 12:431-439.	Score=3.5 or greater
Motifs	A program that searches amino acid sequences for patterns that matched those defined in Prosite.	Bairoch, A. et al. (1997) Nucleic Acids Res. 25:217-221; Wisconsin Package Program Manual, version 9, page M51-59, Genetics Computer Group, Madison, WI.	

What is claimed is:

1. An isolated polypeptide comprising an amino acid sequence selected from the group consisting of:

- 5 a) an amino acid sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, and SEQ ID NO:14.
- b) a naturally occurring amino acid sequence having at least 90% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, and SEQ ID NO:14,
- 10 c) a biologically active fragment of an amino acid sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, and SEQ ID NO:14, and
- 15 d) an immunogenic fragment of an amino acid sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, and SEQ ID NO:14.

- 20 2. An isolated polypeptide of claim 1 selected from the group consisting of SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, and SEQ ID NO:14.

- 25 3. An isolated polynucleotide encoding a polypeptide of claim 1.

4. An isolated polynucleotide encoding a polypeptide of claim 2.

5. An isolated polynucleotide of claim 4 selected from the group consisting of SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, and SEQ ID NO:28.
- 30

6. A recombinant polynucleotide comprising a promoter sequence operably linked to a polynucleotide of claim 3.

- 35 7. A cell transformed with a recombinant polynucleotide of claim 6.

8. A transgenic organism comprising a recombinant polynucleotide of claim 6.

9. A method for producing a polypeptide of claim 1, the method comprising:

- a) culturing a cell under conditions suitable for expression of the polypeptide, wherein said cell is transformed with a recombinant polynucleotide, and said recombinant polynucleotide comprises a promoter sequence operably linked to a polynucleotide encoding the polypeptide of claim 1, and
- b) recovering the polypeptide so expressed.

10. An isolated antibody which specifically binds to a polypeptide of claim 1.

11. An isolated polynucleotide comprising a polynucleotide sequence selected from the group consisting of:

- a) a polynucleotide sequence selected from the group consisting of SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, and SEQ ID NO:28,

- b) a naturally occurring polynucleotide sequence having at least 70% sequence identity to a polynucleotide sequence selected from the group consisting of SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, and SEQ ID NO:28,

- c) a polynucleotide sequence complementary to a),

- d) a polynucleotide sequence complementary to b), and

- e) an RNA equivalent of a)-d).

12. An isolated polynucleotide comprising at least 60 contiguous nucleotides of a polynucleotide of claim 11.

13. A method for detecting a target polynucleotide in a sample, said target polynucleotide having a sequence of a polynucleotide of claim 11, the method comprising:

- a) hybridizing the sample with a probe comprising at least 20 contiguous nucleotides comprising a sequence complementary to said target polynucleotide in the sample, and which probe specifically hybridizes to said target polynucleotide, under conditions whereby a hybridization complex is formed between said probe and said target polynucleotide or fragments thereof, and

- b) detecting the presence or absence of said hybridization complex, and, optionally, if present, the amount thereof.

14. A method of claim 13, wherein the probe comprises at least 60 contiguous nucleotides.

15. A method for detecting a target polynucleotide in a sample, said target polynucleotide having a sequence of a polynucleotide of claim 11, the method comprising:

5 a) amplifying said target polynucleotide or fragment thereof using polymerase chain reaction amplification, and

b) detecting the presence or absence of said amplified target polynucleotide or fragment thereof, and, optionally, if present, the amount thereof.

10 16. A composition comprising an effective amount of a polypeptide of claim 1 and a pharmaceutically acceptable excipient.

17. A composition of claim 16, wherein the polypeptide comprises an amino acid sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, 15 SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, and SEQ ID NO:14.

18. A method for treating a disease or condition associated with decreased expression of functional HYENZ, comprising administering to a patient in need of such treatment the composition 20 of claim 16.

19. A method for screening a compound for effectiveness as an agonist of a polypeptide of claim 1, the method comprising:

25 a) exposing a sample comprising a polypeptide of claim 1 to a compound, and
b) detecting agonist activity in the sample.

20. A composition comprising an agonist compound identified by a method of claim 19 and a pharmaceutically acceptable excipient.

30 21. A method for treating a disease or condition associated with decreased expression of functional HYENZ, comprising administering to a patient in need of such treatment a composition of claim 20.

22. A method for screening a compound for effectiveness as an antagonist of a polypeptide 35 of claim 1, the method comprising:

- a) exposing a sample comprising a polypeptide of claim 1 to a compound, and
- b) detecting antagonist activity in the sample.

23. A composition comprising an antagonist compound identified by a method of claim 22
5 and a pharmaceutically acceptable excipient.

24. A method for treating a disease or condition associated with overexpression of functional
HYENZ, comprising administering to a patient in need of such treatment a composition of claim 23.

10 25. A method of screening for a compound that specifically binds to the polypeptide of claim
1, said method comprising the steps of:

- a) combining the polypeptide of claim 1 with at least one test compound under suitable
conditions, and
- b) detecting binding of the polypeptide of claim 1 to the test compound, thereby identifying a
15 compound that specifically binds to the polypeptide of claim 1.

26. A method of screening for a compound that modulates the activity of the polypeptide of
claim 1, said method comprising:

- a) combining the polypeptide of claim 1 with at least one test compound under conditions
20 permissive for the activity of the polypeptide of claim 1,
- b) assessing the activity of the polypeptide of claim 1 in the presence of the test compound,
and
- c) comparing the activity of the polypeptide of claim 1 in the presence of the test compound
with the activity of the polypeptide of claim 1 in the absence of the test compound, wherein a change
25 in the activity of the polypeptide of claim 1 in the presence of the test compound is indicative of a
compound that modulates the activity of the polypeptide of claim 1.

27. A method for screening a compound for effectiveness in altering expression of a target
polynucleotide, wherein said target polynucleotide comprises a sequence of claim 5, the method
30 comprising:

- a) exposing a sample comprising the target polynucleotide to a compound, and
- b) detecting altered expression of the target polynucleotide.

28. A method for assessing toxicity of a test compound, said method comprising:
35 a) treating a biological sample containing nucleic acids with the test compound;

b) hybridizing the nucleic acids of the treated biological sample with a probe comprising at least 20 contiguous nucleotides of a polynucleotide of claim 11 under conditions whereby a specific hybridization complex is formed between said probe and a target polynucleotide in the biological sample, said target polynucleotide comprising a polynucleotide sequence of a polynucleotide of claim

5 11 or fragment thereof;

c) quantifying the amount of hybridization complex; and

d) comparing the amount of hybridization complex in the treated biological sample with the amount of hybridization complex in an untreated biological sample, wherein a difference in the amount of hybridization complex in the treated biological sample is indicative of toxicity of the test

10 compound.

SEQUENCE LISTING

<110> INCYTE GENOMICS, INC.
 YUE, Henry
 HILLMAN, Jennifer L.
 TANG, Y. Tom
 BAUGHN, Mariah R.
 LU, Dyung Aina M.
 AZIMAZAI, Yalda

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Val Glu Trp Ser Pro	110	Arg Glu Glu Lys	115	Tyr His Leu Arg Gly	120
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Pro Gln Gly Pro Ala	230	Met Thr Trp Ser	235	Met Phe Ala Val Gly	240
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Gln	Tyr	Gln	Lys	Thr	Lys	Leu	Thr	Ile	Leu	Gln	Ser	Leu	Gly	Asp
				350					355					360
Pro	Leu	Tyr	Tyr	Gly	Lys	Ile	Gln	Pro	Cys	Lys	Ala	Asp	Glu	Glu
				365					370					375
Ser	Asp	Ser	Gln	Met	Ser	Pro	Ser	His	Ser	Ser	Thr	Asp	Asp	His
				380					385					390
Ser	Asn	Trp	Phe	Ile	Ile	Gly	Ser	Lys	Thr	Asp	Ala	Glu	Arg	Val
				395					400					405
Val	Asn	Gln	Tyr	Gln	Glu	Leu	Val	Gln	Asn	Glu	Ala	Lys	Cys	Pro
				410					415					420
Val	Lys	Met	Ser	His	Ser	Ser	Ser	Gly	Ser	Ala	Ser	Leu	Ser	Gln
				425					430					435
Val	Ser	Pro	Gly	Lys	Glu	Thr	Asp	Gln	Thr	Glu	Thr	Val	Ser	Val
				440					445					450
Gln	Ser	Ser	Val	Leu	Gly	Lys	Gly	Val	Lys	His	Arg	Pro	Pro	Pro
				455					460					465
Ile	Lys	Leu	Pro	Ser	Ser	Ser	Gly	Asn	Ser	Ser	Ser	Gly	Asn	Tyr
				470					475					480
Phe	Thr	Pro	Gln	Gln	Thr	Ser	Ser	Phe	Leu	Lys	Ser	Pro	Thr	Pro
				485					490					495
Pro	Pro	Ser	Ser	Lys	Pro	Ser	Ser	Ile	Pro	Arg	Lys	Ser	Ser	Val
				500					505					510
Asp	Leu	Asn	Gln	Val	Ser	Met	Leu	Ser	Pro	Ala	Ala	Leu	Ser	Pro
				515					520					525
Ala	Ser	Ser	Ser	Gln	Arg	Thr	Thr	Ala	Thr	Gln	Val	Met	Ala	Asn
				530					535					540
Ser	Ala	Gly	Leu	Asn	Phe	Ile	Asn	Val	Val	Gly	Ser	Val	Cys	Gly
				545					550					555
Ala	Gln	Ala	Leu	Met	Ser	Gly	Ser	Asn	Pro	Met	Leu	Gly	Cys	Asn
				560					565					570
Thr	Gly	Ala	Ile	Thr	Pro	Ala	Gly	Ile	Asn	Leu	Ser	Gly	Leu	Leu
				575					580					585
Pro	Ser	Gly	Gly	Leu	Leu	Pro	Asn	Ala	Leu	Pro	Ser	Ala	Met	Gln
				590					595					600
Ala	Ala	Ser	Gln	Ala	Gly	Val	Pro	Phe	Gly	Leu	Lys	Asn	Thr	Ser
				605					610					615
Ser	Leu	Arg	Pro	Leu	Asn	Leu	Leu	Gln	Leu	Pro	Gly	Gly	Ser	Leu
				620					625					630
Ile	Phe	Asn	Thr	Leu	Gln	Gln	Gln	Gln	Gln	Gln	Leu	Ser	Gln	Phe



Thr	Pro	Gln	Gln	635	Pro	Gln	Gln	Pro	Thr	640	Thr	Cys	Ser	Pro	Gln	645
				650						655						660
Pro	Gly	Glu	Gln	665	Gly	Ser	Glu	Gln	Gly	670	Ser	Thr	Ser	Gln	Glu	675
Ala	Leu	Ser	Ala	680	Gln	Gln	Ala	Ala	Val	685	Ile	Asn	Leu	Thr	Gly	690
Gly	Ser	Phe	Met	695	Gln	Ser	Gln	Ala	Ala	700	Ala	Val	Ala	Ile	Leu	705
Ala	Ser	Asn	Gly	710	Tyr	Gly	Ser	Ser	Ser	715	Ser	Thr	Asn	Ser	Ser	720
Thr	Ser	Ser	Ser	725	Ala	Tyr	Arg	Gln	Pro	730	Val	Lys	Lys			

<210> 7
 <211> 343
 <212> PRT
 <213> Homo sapiens

<220>
 <221> misc_feature
 <223> Incyte ID No: 2612754CD1

<400> 7

Met	Ala	Gly	Gly	Arg	Pro	His	Leu	Lys	Arg	Ser	Phe	Ser	Ile	Ile		
1				5					10					15		
Pro	Cys	Phe	Val	Phe	Val	Glu	Ser	Val	Leu	Leu	Gly	Ile	Val	Ile		
				20					25					30		
Leu	Leu	Ala	Tyr	Arg	Leu	Glu	Phe	Thr	Asp	Thr	Phe	Pro	Val	His		
				35					40					45		
Thr	Gln	Gly	Phe	Phe	Cys	Tyr	Asp	Ser	Thr	Tyr	Ala	Lys	Pro	Tyr		
				50					55					60		
Pro	Gly	Pro	Glu	Ala	Ala	Ser	Arg	Val	Pro	Pro	Ala	Leu	Val	Tyr		
				65					70					75		
Ala	Leu	Val	Thr	Ala	Gly	Pro	Thr	Leu	Thr	Ile	Leu	Leu	Gly	Glu		
				80					85					90		
Leu	Ala	Arg	Pro	Phe	Phe	Pro	Ala	Pro	Pro	Ser	Ala	Val	Pro	Val		
				95					100					105		
Ile	Gly	Glu	Ser	Thr	Ile	Val	Ser	Gly	Ala	Cys	Cys	Arg	Phe	Ser		
				110					115					120		
Pro	Pro	Val	Arg	Arg	Leu	Val	Arg	Phe	Leu	Gly	Val	Tyr	Ser	Phe		
				125					130					135		
Gly	Leu	Phe	Thr	Thr	Thr	Ile	Phe	Ala	Asn	Ala	Gly	Gln	Val	Val		
				140					145					150		
Thr	Gly	Asn	Pro	Thr	Pro	His	Phe	Leu	Ser	Val	Cys	Arg	Pro	Asn		
				155					160					165		
Tyr	Thr	Ala	Leu	Gly	Cys	Leu	Pro	Pro	Ser	Pro	Asp	Arg	Pro	Gly		
				170					175					180		
Pro	Asp	Arg	Phe	Val	Thr	Asp	Gln	Gly	Ala	Cys	Ala	Gly	Ser	Pro		
				185					190					195		
Ser	Leu	Val	Ala	Ala	Ala	Arg	Arg	Ala	Phe	Pro	Cys	Lys	Asp	Ala		
				200					205					210		
Ala	Leu	Cys	Ala	Tyr	Ala	Val	Thr	Tyr	Thr	Ala	Met	Tyr	Val	Thr		
				215					220					225		
Leu	Val	Phe	Arg	Val	Lys	Gly	Ser	Arg	Leu	Val	Lys	Pro	Ser	Leu		
				230					235					240		
Cys	Leu	Ala	Leu	Leu	Cys	Pro	Ala	Phe	Leu	Val	Gly	Val	Val	Arg		
				245					250					255		
Val	Ala	Glu	Tyr	Arg	Asn	His	Trp	Ser	Asp	Val	Leu	Ala	Gly	Phe		
				260					265					270		
Leu	Thr	Gly	Ala	Ala	Ile	Ala	Thr	Phe	Leu	Val	Thr	Cys	Val	Val		
				275					280					285		
His	Asn	Phe	Gln	Ser	Arg	Pro	Pro	Ser	Gly	Arg	Ser	Val	Ser	Pro		
				290					295					300		



Trp	Glu	Asp	Leu	Gly	Gln	Ala	Pro	Thr	Met	Asp	Ser	Pro	Leu	Glu
				305					310					315
Lys	Asn	Pro	Arg	Ser	Ala	Gly	Arg	Ile	Arg	His	Arg	His	Gly	Ser
				320					325					330
Pro	His	Pro	Ser	Arg	Arg	Thr	Ala	Pro	Ala	Val	Ala	Thr		
				335					340					

<210> 8

<211> 717

<212> PRT

<213> Homo sapiens

<220>

<221> misc_feature

<223> Incyte ID No: 2616646CD1

<400> 8

Met	Arg	Arg	Ser	Pro	Pro	Ser	Leu	Arg	Leu	Arg	Leu	Ser	Ala	Asp
1				5					10					15
Asn	Leu	Val	Ala	Ala	Ser	Gly	Gly	Cys	Trp	Phe	Val	Leu	Gly	Glu
				20					25					30
Arg	Arg	Ala	Gly	Ser	Leu	Leu	Ser	Ala	Ser	Tyr	Gly	Thr	Phe	Ala
				35					40					45
Met	Pro	Gly	Met	Val	Leu	Phe	Gly	Arg	Arg	Trp	Ala	Ile	Ala	Ser
				50					55					60
Asp	Asp	Leu	Val	Phe	Pro	Gly	Phe	Phe	Glu	Leu	Val	Val	Arg	Val
				65					70					75
Leu	Trp	Trp	Ile	Gly	Ile	Leu	Thr	Leu	Tyr	Leu	Met	His	Arg	Gly
				80					85					90
Lys	Leu	Asp	Cys	Ala	Gly	Gly	Ala	Leu	Leu	Ser	Ser	Tyr	Leu	Ile
				95					100					105
Val	Leu	Met	Ile	Leu	Leu	Ala	Val	Val	Ile	Cys	Thr	Val	Ser	Ala
				110					115					120
Ile	Met	Cys	Val	Ser	Met	Arg	Gly	Thr	Ile	Cys	Asn	Pro	Gly	Pro
				125					130					135
Arg	Lys	Ser	Met	Ser	Lys	Leu	Leu	Tyr	Ile	Arg	Leu	Ala	Leu	Phe
				140					145					150
Phe	Pro	Glu	Met	Val	Trp	Ala	Ser	Leu	Gly	Ala	Ala	Trp	Val	Ala
				155					160					165
Asp	Gly	Val	Gln	Cys	Asp	Arg	Thr	Val	Val	Asn	Gly	Ile	Ile	Ala
				170					175					180
Thr	Val	Val	Val	Ser	Trp	Ile	Ile	Ile	Ala	Ala	Thr	Val	Val	Ser
				185					190					195
Ile	Ile	Ile	Val	Phe	Asp	Pro	Leu	Gly	Gly	Lys	Met	Ala	Pro	Tyr
				200					205					210
Ser	Ser	Ala	Gly	Pro	Ser	His	Leu	Asp	Ser	His	Asp	Ser	Ser	Gln
				215					220					225
Leu	Leu	Asn	Gly	Leu	Lys	Thr	Ala	Ala	Thr	Ser	Val	Trp	Glu	Thr
				230					235					240
Arg	Ile	Lys	Leu	Leu	Cys	Cys	Cys	Ile	Gly	Lys	Asp	Asp	His	Thr
				245					250					255
Arg	Val	Ala	Phe	Ser	Ser	Thr	Ala	Glu	Leu	Phe	Ser	Thr	Tyr	Phe
				260					265					270
Ser	Asp	Thr	Asp	Leu	Val	Pro	Ser	Asp	Ile	Ala	Ala	Gly	Leu	Ala
				275					280					285
Leu	Leu	His	Gln	Gln	Gln	Asp	Asn	Ile	Arg	Asn	Asn	Gln	Glu	Pro
				290					295					300
Ala	Gln	Val	Val	Cys	His	Ala	Pro	Gly	Ser	Ser	Gln	Glu	Ala	Asp
				305					310					315
Leu	Asp	Ala	Glu	Leu	Glu	Asn	Cys	His	His	Tyr	Met	Gln	Phe	Ala
				320					325					330
Ala	Ala	Ala	Tyr	Gly	Trp	Pro	Leu	Tyr	Ile	Tyr	Arg	Asn	Pro	Leu
				335					340					345
Thr	Gly	Leu	Cys	Arg	Ile	Gly	Gly	Asp	Cys	Cys	Arg	Ser	Arg	Thr



Thr Asp Tyr Asp	Leu Val Gly Gly Asp	Gln Leu Asn Cys His	Phe
350	355	360	
365	370	375	
Gly Ser Ile Leu	His Thr Thr Gly Leu	Gln Tyr Arg Asp Phe	Ile
380	385	390	
His Val Ser Phe	His Asp Lys Val Tyr	Glu Leu Pro Phe Leu	Val
395	400	405	
Ala Leu Asp His	Arg Lys Glu Ser Val	Val Val Ala Val Arg	Gly
410	415	420	
Thr Met Ser Leu	Gln Asp Val Leu Thr	Asp Leu Ser Ala Glu	Ser
425	430	435	
Glu Val Leu Asp	Val Glu Cys Glu Val	Gln Asp Arg Leu Ala	His
440	445	450	
Lys Gly Ile Ser	Gln Ala Ala Arg Tyr	Val Tyr Gln Arg Leu	Ile
455	460	465	
Asn Asp Gly Ile	Leu Ser Gln Ala Phe	Ser Ile Ala Pro Glu	Tyr
470	475	480	
Arg Leu Val Ile	Val Gly His Ser Leu	Gly Gly Gly Ala Ala	Ala
485	490	495	
Leu Leu Ala Thr	Met Leu Arg Ala Ala	Tyr Pro Gln Val Arg	Cys
500	505	510	
Tyr Ala Phe Ser	Pro Pro Arg Gly Leu	Trp Ser Lys Ala Leu	Gln
515	520	525	
Glu Tyr Ser Gln	Ser Phe Ile Val Ser	Leu Val Leu Gly Lys	Asp
530	535	540	
Val Ile Pro Arg	Leu Ser Val Thr Asn	Leu Glu Asp Leu Lys	Arg
545	550	555	
Arg Ile Leu Arg	Val Val Ala His Cys	Asn Lys Pro Lys Tyr	Lys
560	565	570	
Ile Leu Leu His	Gly Leu Trp Tyr Glu	Leu Phe Gly Gly Asn	Pro
575	580	585	
Asn Asn Leu Pro	Thr Glu Leu Asp Gly	Gly Asp Gln Glu Val	Leu
590	595	600	
Thr Gln Pro Leu	Leu Gly Glu Gln Ser	Leu Leu Thr Arg Trp	Ser
605	610	615	
Pro Ala Tyr Ser	Phe Ser Ser Asp Ser	Pro Leu Asp Ser Ser	Pro
620	625	630	
Lys Tyr Pro Pro	Leu Tyr Pro Pro Gly	Arg Ile Ile His Leu	Gln
635	640	645	
Glu Glu Gly Ala	Ser Gly Arg Phe Gly	Cys Cys Ser Ala Ala	His
650	655	660	
Tyr Ser Ala Lys	Trp Ser His Glu Ala	Glu Phe Ser Lys Ile	Leu
665	670	675	
Ile Gly Pro Lys	Met Leu Thr Asp His	Met Pro Asp Ile Leu	Met
680	685	690	
Arg Ala Leu Asp	Ser Val Val Ser Asp	Arg Ala Ala Cys Val	Ser
695	700	705	
Cys Pro Ala Gln	Gly Val Ser Ser Val	Asp Val Ala	
710	715		

<210> 9
 <211> 236
 <212> PRT
 <213> Homo sapiens

<220>
 <221> misc_feature
 <223> Incyte ID No: 2625111CD1

<400> 9
 Met Leu Pro Asp Cys Leu Ser Ala Glu Gly Glu Leu Arg Cys Arg
 1 5 10 15
 Arg Leu Leu Ala Gly Ala Thr Ala Arg Leu Arg Ala Arg Pro Ala
 20 25 30



Ser	Ala	Ala	Val	Leu	Val	Pro	Leu	Cys	Ser	Val	Arg	Gly	Val	Pro
				35					40					45
Ala	Leu	Leu	Tyr	Thr	Leu	Arg	Ser	Ser	Arg	Leu	Thr	Gly	Arg	His
				50					55					60
Lys	Gly	Asp	Val	Ser	Phe	Pro	Gly	Gly	Lys	Cys	Asp	Pro	Ala	Asp
				65					70					75
Gln	Asp	Val	Val	His	Thr	Ala	Leu	Arg	Glu	Thr	Arg	Glu	Glu	Leu
				80					85					90
Gly	Leu	Ala	Val	Pro	Glu	Glu	His	Val	Trp	Gly	Leu	Leu	Arg	Pro
				95					100					105
Val	Tyr	Asp	Pro	Gln	Lys	Ala	Thr	Val	Val	Pro	Val	Leu	Ala	Gly
				110					115					120
Val	Gly	Pro	Leu	Asp	Pro	Gln	Ser	Leu	Arg	Pro	Asn	Ser	Glu	Glu
				125					130					135
Val	Asp	Glu	Val	Phe	Ala	Leu	Pro	Leu	Ala	His	Leu	Leu	Gln	Thr
				140					145					150
Gln	Asn	Gln	Gly	Tyr	Thr	His	Phe	Cys	Arg	Gly	Gly	His	Phe	Arg
				155					160					165
Tyr	Thr	Leu	Pro	Val	Phe	Leu	His	Gly	Pro	His	Arg	Val	Trp	Gly
				170					175					180
Leu	Thr	Ala	Val	Ile	Thr	Glu	Phe	Ala	Leu	Gln	Leu	Leu	Ala	Pro
				185					190					195
Gly	Thr	Tyr	Gln	Pro	Arg	Leu	Ala	Gly	Leu	Thr	Cys	Ser	Gly	Ala
				200					205					210
Glu	Gly	Leu	Ala	Arg	Pro	Lys	Gln	Pro	Leu	Ala	Ser	Pro	Cys	Gln
				215					220					225
Ala	Ser	Ser	Thr	Pro	Gly	Leu	Asn	Lys	Gly	Leu				
				230					235					

<210> 10
 <211> 386
 <212> PRT
 <213> Homo sapiens

<220>
 <221> misc_feature
 <223> Incyte ID No: 2724525CD1

<400> 10
 Met Ser Ala Leu Glu Lys Ser Met His Leu Gly Arg Leu Pro Ser
 1 5 10 15
 Arg Pro Pro Leu Pro Gly Ser Gly Gly Ser Gln Ser Gly Ala Lys
 20 25 30
 Met Arg Met Gly Pro Gly Arg Lys Arg Asp Phe Ser Pro Val Pro
 35 40 45
 Trp Ser Gln Tyr Phe Glu Ser Met Glu Asp Val Glu Val Glu Asn
 50 55 60
 Glu Thr Gly Lys Asp Thr Phe Arg Val Tyr Lys Ser Gly Ser Glu
 65 70 75
 Gly Pro Val Leu Leu Leu Leu His Gly Gly Gly His Ser Ala Leu
 80 85 90
 Ser Trp Ala Val Phe Thr Ala Ala Ile Ile Ser Arg Val Gln Cys
 95 100 105
 Arg Ile Val Ala Leu Asp Leu Arg Ser His Gly Glu Thr Lys Val
 110 115 120
 Lys Asn Pro Glu Asp Leu Ser Ala Glu Thr Met Ala Lys Asp Val
 125 130 135
 Gly Asn Val Val Glu Ala Met Tyr Gly Asp Leu Pro Pro Pro Ile
 140 145 150
 Met Leu Ile Gly His Ser Met Gly Gly Ala Ile Ala Val His Thr
 155 160 165
 Ala Ser Ser Asn Leu Val Pro Ser Leu Leu Gly Leu Cys Met Ile
 170 175 180
 Asp Val Val Glu Gly Thr Ala Met Asp Ala Leu Asn Ser Met Gln



Asn Phe Leu Arg	185	Arg Pro Lys Thr	190	Phe Lys Ser Leu Glu Asn	195
	200		205		210
Ala Ile Glu Trp	215	Ser Val Lys Ser Gly	220	Gln Ile Arg Asn Leu Glu	225
Ser Ala Arg Val	230	Ser Met Val Gly Gln	235	Val Lys Gln Cys Glu Gly	240
Ile Thr Ser Pro	245	Glu Gly Ser Lys Ser	250	Ile Val Glu Gly Ile Ile	255
Glu Glu Glu Glu	260	Glu Asp Glu Glu Gly	265	Ser Glu Ser Ile Ser Lys	270
Arg Lys Lys Glu	275	Asp Asp Met Glu Thr	280	Lys Lys Asp His Pro Tyr	285
Thr Trp Arg Ile	290	Glu Leu Ala Lys Thr	295	Glu Lys Tyr Trp Asp Gly	300
Trp Phe Arg Gly	305	Leu Ser Asn Leu Phe	310	Leu Ser Cys Pro Ile Pro	315
Lys Leu Leu Leu	320	Leu Ala Gly Val Asp	325	Arg Leu Asp Lys Asp Leu	330
Thr Ile Gly Gln	335	Met Gln Gly Lys Phe	340	Gln Met Gln Val Leu Pro	345
Gln Cys Gly His	350	Ala Val His Glu Asp	355	Ala Pro Asp Lys Val Ala	360
Glu Ala Val Ala	365	Thr Phe Leu Ile Arg	370	His Arg Phe Ala Glu Pro	375
Ile Gly Gly Phe	380	Gln Cys Val Phe Pro	385	Gly Cys	

<210> 11
 <211> 522
 <212> PRT
 <213> Homo sapiens

<220>
 <221> misc_feature
 <223> Incyte ID No: 2824691CD1

<400> 11

Met Val Arg Ser Gly	1	Lys Asn Gly Asp Leu	10	His Leu Lys Gln Ile	15
Ala Tyr Tyr Lys Arg	20	Thr Gly Glu Tyr His	25	Ser Thr Thr Leu Pro	30
Ser Glu Arg Ser Gly	35	Ile Arg Arg Ala Ala	40	Lys Lys Phe Val Phe	45
Lys Glu Lys Lys Leu	50	Phe Tyr Val Gly Lys	55	Asp Arg Lys Gln Asn	60
Arg Leu Val Ile Val	65	Ser Glu Glu Glu Lys	70	Lys Lys Val Leu Arg	75
Glu Cys His Glu Asn	80	Asp Ser Gly Ala His	85	His Gly Ile Ser Arg	90
Thr Leu Thr Leu Val	95	Glu Ser Asn Tyr Tyr	100	Trp Thr Ser Val Thr	105
Asn Asp Val Lys Gln	110	Trp Val Tyr Ala Cys	115	Gln His Cys Gln Val	120
Ala Lys Asn Thr Val	125	Ile Val Ala Pro Lys	130	Gln His Leu Leu Lys	135
Val Glu Asn Pro Trp	140	Ser Leu Val Thr Val	145	Asp Leu Met Gly Pro	150
Phe His Thr Ser Asn	155	Arg Ser His Val Tyr	160	Ala Ile Ile Met Thr	165
Asp Leu Phe Thr Lys	170	Trp Ile Val Ile Leu	175	Pro Leu Cys Asp Val	180
Ser Ala Ser Glu Val	185	Ser Lys Ala Ile Ile	190	Asn Ile Phe Phe Leu	195



Tyr	Gly	Pro	Pro	Gln	Lys	Ile	Ile	Met	Asp	Gln	Arg	Asp	Glu	Phe	200	205	210
Ile	Gln	Gln	Ile	Asn	Ile	Glu	Leu	Tyr	Arg	Leu	Phe	Gly	Ile	Lys	215	220	225
Gln	Ile	Val	Ile	Ser	His	Thr	Ser	Gly	Thr	Val	Asn	Pro	Met	Glu	230	235	240
Ser	Thr	Pro	Asn	Thr	Ile	Lys	Ala	Phe	Leu	Ser	Lys	His	Cys	Ala	245	250	255
Asp	His	Pro	Asn	Asn	Trp	Asp	Asp	His	Leu	Ser	Ala	Val	Ser	Phe	260	265	270
Ala	Phe	Asn	Val	Thr	His	Leu	Glu	Pro	Thr	Lys	Asn	Thr	Pro	Tyr	275	280	285
Phe	Gln	Met	Phe	Ser	Arg	Asn	Pro	Tyr	Met	Pro	Glu	Thr	Ser	Asp	290	295	300
Ser	Leu	His	Glu	Val	Asp	Gly	Asp	Asn	Thr	Ser	Met	Phe	Ala	Lys	305	310	315
Ile	Leu	Asp	Ala	Ile	Lys	Glu	Ala	Asp	Lys	Ile	Met	Glu	Asn	Lys	320	325	330
Thr	Thr	Ser	Leu	Gly	Gln	Met	Glu	Asn	Asn	Asn	Leu	Asp	Glu	Leu	335	340	345
Asn	Lys	Ser	Lys	Ile	Ile	Val	Lys	Lys	Lys	Pro	Lys	Gln	Leu	Asn	350	355	360
Pro	Phe	His	Leu	Lys	Val	Gly	His	Glu	Val	Leu	Arg	Gln	Arg	Lys	365	370	375
Asn	Trp	Trp	Lys	Asp	Gly	Arg	Phe	Gln	Ser	Glu	Trp	Val	Gly	Pro	380	385	390
Cys	Val	Ile	Asp	Tyr	Ile	Thr	Glu	Ser	Gly	Cys	Ala	Val	Leu	Arg	395	400	405
Asp	Asn	Thr	Gly	Val	Arg	Leu	Lys	Arg	Pro	Ile	Lys	Met	Ser	His	410	415	420
Leu	Lys	Pro	Tyr	Ile	Arg	Glu	Ser	Ser	Glu	Gln	Glu	Ser	Leu	Tyr	425	430	435
Leu	Leu	Gln	Gly	Ser	Val	Val	Ala	Asp	His	Asp	Tyr	Ile	Gly	Leu	440	445	450
Pro	Glu	Ile	Pro	Ile	Gly	Ala	Tyr	Gln	Ala	Asn	Ile	Leu	Val	Glu	455	460	465
Asp	Ala	Thr	Ile	Gly	Ile	Val	Asp	Asn	Glu	Leu	Leu	Thr	Ser	Ser	470	475	480
Lys	Asp	Arg	Glu	Leu	Leu	Glu	Tyr	Arg	Asn	Thr	Lys	Ile	Ser	Pro	485	490	495
Leu	Ile	Asp	Asp	His	Ser	Ser	Leu	Glu	Lys	Gln	Thr	Phe	Ser	Leu	500	505	510
Leu	Asp	Ser	Ser	Asn	Gln	Val	Leu	Glu	Tyr	Leu	Ser				515	520	

<210> 12
 <211> 420
 <212> PRT
 <213> Homo sapiens

<220>
 <221> misc_feature
 <223> Incyte ID No: 4722794CD1

<400> 12
 Met Thr Met Glu Lys Gly Met Ser Ser Gly Glu Gly Leu Pro Ser
 1 5 10 15
 Arg Ser Ser Gln Val Ser Ala Gly Lys Ile Thr Ala Lys Glu Leu
 20 25 30
 Glu Thr Lys Gln Ser Tyr Lys Glu Lys Arg Gly Gly Phe Val Leu
 35 40 45
 Val His Ala Gly Ala Gly Tyr His Ser Glu Ser Lys Ala Lys Glu
 50 55 60
 Tyr Lys His Val Cys Lys Arg Ala Cys Gln Lys Ala Ile Glu Lys



65	70	75
Leu Gln Ala Gly Ala	Leu Ala Thr Asp Ala	Val Thr Ala Ala Leu
80	85	90
Val Glu Leu Glu Asp	Ser Pro Phe Thr Asn	Ala Gly Met Gly Ser
95	100	105
Asn Leu Asn Leu Leu	Gly Glu Ile Glu Cys	Asp Ala Ser Ile Met
110	115	120
Asp Gly Lys Ser Leu	Asn Phe Gly Ala Val	Gly Ala Leu Ser Gly
125	130	135
Ile Lys Asn Pro Val	Ser Val Ala Asn Arg	Leu Leu Cys Glu Gly
140	145	150
Gln Lys Gly Lys Leu	Ser Ala Gly Arg Ile	Pro Pro Cys Phe Leu
155	160	165
Val Gly Glu Gly Ala	Tyr Arg Trp Ala Val	Asp His Gly Ile Pro
170	175	180
Ser Cys Pro Pro Asn	Ile Met Thr Thr Arg	Phe Ser Leu Ala Ala
185	190	195
Phe Lys Arg Asn Lys	Arg Lys Leu Glu Leu	Ala Glu Arg Val Asp
200	205	210
Thr Asp Phe Met Gln	Leu Lys Lys Arg Arg	Gln Ser Ser Glu Lys
215	220	225
Glu Asn Asp Ser Gly	Thr Leu Asp Thr Val	Gly Ala Val Val Val
230	235	240
Asp His Glu Gly Asn	Val Ala Ala Ala Val	Ser Ser Gly Gly Leu
245	250	255
Ala Leu Lys His Pro	Gly Arg Val Gly Gln	Ala Ala Leu Tyr Gly
260	265	270
Cys Gly Cys Trp Ala	Glu Asn Thr Gly Ala	His Asn Pro Tyr Ser
275	280	285
Thr Ala Val Ser Thr	Ser Gly Cys Gly Glu	His Leu Val Arg Thr
290	295	300
Ile Leu Ala Arg Glu	Cys Ser His Ala Leu	Gln Ala Glu Asp Ala
305	310	315
His Gln Ala Leu Leu	Glu Thr Met Gln Asn	Lys Phe Ile Ser Ser
320	325	330
Pro Phe Leu Ala Ser	Glu Asp Gly Val Leu	Gly Gly Val Ile Val
335	340	345
Leu Arg Ser Cys Arg	Cys Ser Ala Glu Pro	Asp Ser Ser Gln Asn
350	355	360
Lys Gln Thr Leu Leu	Val Glu Phe Leu Trp	Ser His Thr Thr Glu
365	370	375
Ser Met Cys Val Gly	Tyr Met Ser Ala Gln	Asp Gly Lys Ala Lys
380	385	390
Thr His Ile Ser Arg	Leu Pro Pro Gly Ala	Val Ala Gly Gln Ser
395	400	405
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410	415	420

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 <213> Homo sapiens

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 Lys Arg Asn Gly Met Ser Gly Tyr Tyr Gly Val Ser Leu Ala Asp
 35 40 45



Trp	Val	Cys	Leu	Ala	Gln	His	Glu	Ser	Asn	Tyr	Asn	Thr	Gln	Ala	
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Arg	Asn	Tyr	Asn	Pro	Gly	Asp	Gln	Ser	Thr	Asp	Tyr	Gly	Ile	Phe	
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Ala	Lys	Asn	Ala	Cys	Gly	Ile	Pro	Cys	Ser	Ala	Leu	Leu	Gln	Asp	
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Asp	Ile	Thr	Ala	Ala	Ile	Gln	Cys	Ala	Lys	Arg	Val	Val	Arg	Asp	
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Pro	Gln	Gly	Ile	Arg	Ala	Trp	Val	Ala	Trp	Gln	Arg	His	Cys	Lys	
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Gln	Cys	Thr	Ser	Thr	Gln	Leu	Thr	Leu	Ser	Leu	Ser	His	Cys	Gly	
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Ser	Ser	Tyr	Gly	Glu	Gly	Pro	Thr	Ser	Leu	Leu	Ser	Pro	Gln	Asn	
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Lys	Cys	Asp	Val	Leu	Asn	Arg	Gly	Phe	Ser	Gly	Tyr	Asn	Thr	Arg	
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Trp	Ala	Lys	Ile	Ile	Leu	Pro	Arg	Leu	Ile	Arg	Lys	Gly	Asn	Ser	
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Glu	Tyr	Ala	Ala	Asn	Leu	Lys	Ser	Met	Val	Gln	Tyr	Leu	Lys	Ser	
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Val	Asp	Ile	Pro	Glu	Asn	Arg	Val	Ile	Leu	Ile	Thr	Pro	Thr	Pro	
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Lys	Leu	Asn	Arg	Leu	Asn	Ser	Val	Val	Gly	Glu	Tyr	Ala	Asn	Ala	
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Asp	Gly	Leu	His	Leu	Ser	Pro	Lys	Gly	Asn	Glu	Phe	Leu	Phe	Ser	
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His	Leu	Trp	Pro	Leu	Ile	Glu	Lys	Lys	Val	Ser	Ser	Leu	Pro	Leu	
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Leu	Leu	Pro	Tyr	Trp	Arg	Asp	Val	Ala	Glu	Ala	Lys	Pro	Glu	Leu	
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<212> DNA
<213> Homo sapiens

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PATENT COOPERATION TREATY

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

From the INTERNATIONAL BUREAU

To:

Commissioner
 US Department of Commerce
 United States Patent and Trademark
 Office, PCT
 2011 South Clark Place Room
 CP2/5C24
 Arlington, VA 22202
 ETATS-UNIS D'AMERIQUE
 in its capacity as elected Office

Date of mailing (day/month/year) 10 July 2001 (10.07.01)	
International application No. PCT/US00/24107	Applicant's or agent's file reference PF-0738 PCT
International filing date (day/month/year) 31 August 2000 (31.08.00)	Priority date (day/month/year) 01 September 1999 (01.09.99)
Applicant YUE, Henry et al	

1. The designated Office is hereby notified of its election made:

☒ in the demand filed with the International Preliminary Examining Authority on:
 27 March 2001 (27.03.01)

☐ in a notice effecting later election filed with the International Bureau on:

2. The election ☒ was
☐ was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland	Authorized officer Claudio Borton
Facsimile No.: (41-22) 740.14.35	Telephone No.: (41-22) 338.83.38



PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference PF-0738 PCT	FOR FURTHER ACTION see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.	
International application No. PCT/US 00/ 24107	International filing date (day/month/year) 31/08/2000	(Earliest) Priority Date (day/month/year) 01/09/1999
Applicant INCYTE GENOMICS, INC.		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 6 sheets.

☐ It is also accompanied by a copy of each prior art document cited in this report.

1. Basis of the report

a. With regard to the **language**, the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

☐ the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :

☒ contained in the international application in written form.

☒ filed together with the international application in computer readable form.

☐ furnished subsequently to this Authority in written form.

☐ furnished subsequently to this Authority in computer readable form.

☐ the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

☐ the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. ☒ **Certain claims were found unsearchable** (See Box I).

3. ☒ **Unity of invention is lacking** (see Box II).

4. With regard to the **title**,

☒ the text is approved as submitted by the applicant.

☐ the text has been established by this Authority to read as follows:

5. With regard to the **abstract**,

☒ the text is approved as submitted by the applicant.

☐ the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No.

☐ as suggested by the applicant.

☐ because the applicant failed to suggest a figure.

☐ because this figure better characterizes the invention.

☐ None of the figures.



1

2

3

4

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 00/24107

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Although claim 18 is directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the composition.
2. ☒ Claims Nos.: 20, 21, 23, 24
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
see FURTHER INFORMATION sheet PCT/ISA/210
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1-28 partially

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.



FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: 1-28 partially

Polypeptide of sequence SEQ ID NO:2 and encoding polynucleotide of SEQ ID NO:16, fragments, analogs and applications thereof.

2. Claims: 1-28 partially

Polypeptide of sequence SEQ ID NO:3 and encoding polynucleotide of SEQ ID NO:17, fragments, analogs and applications thereof.

3. Claims: 1-28 partially

Polypeptide of sequence SEQ ID NO:4 and encoding polynucleotide of SEQ ID NO:18, fragments, analogs and applications thereof.

4. Claims: 1-28 partially

Polypeptide of sequence SEQ ID NO:5 and encoding polynucleotide of SEQ ID NO:19, fragments, analogs and applications thereof.

5. Claims: 1-28 partially

Polypeptide of sequence SEQ ID NO:6 and encoding polynucleotide of SEQ ID NO:20, fragments, analogs and applications thereof.

6. Claims: 1-28 partially

Polypeptide of sequence SEQ ID NO:7 and encoding polynucleotide of SEQ ID NO:21, fragments, analogs and applications thereof.

7. Claims: 1-28 partially

Polypeptide of sequence SEQ ID NO:8 and encoding polynucleotide of SEQ ID NO:22, fragments, analogs and applications thereof.

8. Claims: 1-28 partially

Polypeptide of sequence SEQ ID NO:9 and encoding



FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

polynucleotide of SEQ ID NO:23, fragments, analogs and applications thereof.

9. Claims: 1-28 partially

Polypeptide of sequence SEQ ID NO:11 and encoding polynucleotide of SEQ ID NO:25, fragments, analogs and applications thereof.

10. Claims: 1-28 partially

Polypeptide of sequence SEQ ID NO:12 and encoding polynucleotide of SEQ ID NO:26, fragments, analogs and applications thereof.

11. Claims: 1-28 partially

Polypeptide of sequence SEQ ID NO:13 and encoding polynucleotide of SEQ ID NO:27, fragments, analogs and applications thereof.

12. Claims: 1-28 partially

Polypeptide of sequence SEQ ID NO:14 and encoding polynucleotide of SEQ ID NO:28, fragments, analogs and applications thereof.



FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Claims Nos.: 20, 21, 23, 24

Present claims 20, 21, 23 and 24 relate to a compound defined by reference to a desirable characteristic or property, namely agonist or antagonist activity towards the polypeptide of claim 1. The claims cover all compounds having this characteristic or property, whereas the application does not provide support within the meaning of Article 6 PCT and/or disclosure within the meaning of Article 5 PCT for any specific example of such compounds. In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible. Consequently, no search has been carried out for claims 20, 21, 23 and 24.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.



PGPUS 00/24107

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7	C12N15/55	C12N9/14	C12N1/21	A01K67/027	C07K16/40
	C1201/68	A61K38/46	G01N33/68		

IPC 7 C12N A01K C07K C12Q G01N A61K

EMBL, EPO-Internal, WPI Data, BIOSIS, MEDLINE, CHEM ABS Data

X	DATABASE EMBL [Online] Entry AF015416, 26 August 1997 (1997-08-26) EVANS G.A. ET AL.: "95 kb from four overlapping human chromosome 11p15.5 cosmids" XP002159698 nucleotides 78880-80130	11-15
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MONTERO LOPEZ B.



The demand must be filed for the authority competent to grant a preliminary examination. The authority indicated by the applicant on the line IPEA/US

PCT

CHAPTER II

DEMAND

JC18 Rec'd PCT/PTO 27 MAR 2001

under Article 31 of the Patent Cooperation Treaty:
The undersigned requests that the international application specified below be the subject of international preliminary examination according to the Patent Cooperation Treaty and hereby elects all eligible States (except where otherwise indicated).

For International Preliminary Examination Authority use only

Identification of IPEA/US	IPEA/US 27 MAR 2001
	Date of receipt of DEMAND (27.03.01)

Box No. I IDENTIFICATION OF THE INTERNATIONAL APPLICATION		Applicant's or agent's file reference PF-0733 PCT	
International application No. PCT/US00/24107	International filing date (day/month/year) 31 August 2000 (31.08.00)	(Earliest) Priority date (day/month/year) 01 September 1999 (01.09.99)	
Title of invention HUMAN HYDROLYTIC ENZYMES			

Box No. II APPLICANT(S)	
Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.) INCYTE GENOMICS, INC. 3160 Porter Drive Palo Alto, California 94304 United States of America	Telephone No.: (650) 855-0555 Facsimile No.: (650) 845-4166 Teleprinter No.:
State designating agent (name and address) US	State designating agent (name and address) US
Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.) YUIE Henry	



Continuation of Box No. 11 (APPLICANTS)

If none of the following sub-boxes is used, this sheet is not to be included in the demand.

Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)

TANG, Y. Tom
 4230 Ranwick Court
 San Jose, California 95118
 United States of America

State (that is, country) of nationality:

US

State (that is, country) of residence:

US

Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)

BAUGHN, Mariah R.
 14244 Santiago Road
 San Leandro, California 94577
 United States of America

State (that is, country) of nationality:

US

State (that is, country) of residence:

US

Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)

L.J. Dyoung Anna M.
 233 Coy Drive (current address)
 San Jose, California 95123
 United States of America

55 Park Belmont Place (old address)
 San Jose, California 96136
 United States of America



Box No. III AGENT OR COMMON REPRESENTATIVE; OR ADDRESS FOR CORRESPONDENCE

By check all of the following: ☒ Agent ☐ Common representative



The undersigned is/are the agent(s) for the applicant in the international phase of the application.



is hereby appointed and any earlier appointment of (an) agent(s)/common representative is hereby revoked.



is hereby appointed, specifically for the procedure before the International Preliminary Examining Authority, in addition to the agent(s)/common representative appointed earlier.

Name and address: (Family name followed by given name, for a legal entity, full official)
(The address must include postal code and name of country.)

HAMLET-COX, Diana; BILLINGS, Lucy J.; CERRONE, Michael C.;
MURRY, Lynn E.; STREETER, David G.; SATHER, Susan K.; WANG, Peng
Ben; KASER, Matthew R.; TURNER, Christopher
Incyte Genomics, Inc.
3150 Porter Drive
Palo Alto, California 94301
United States of America

Telephone No.:

(650) 855-0555

Facsimile No.:

(650) 845-4166

Teleprinter No.:



Address for correspondence: Mark this check-box where no agent or common representative is/has been appointed and the space above is used instead to indicate a special address to which correspondence should be sent.

Box No. IV BASIS FOR INTERNATIONAL PRELIMINARY EXAMINATION

Statement concerning amendments:*

1. The applicant wishes the international preliminary examination to start on the basis of:



the international application as originally filed

the description



as originally filed



as amended under Article 34

the claims



as originally filed



as amended under Article 19 (together with any accompanying statement)



as amended under Article 34

the drawings



as originally filed



as amended under Article 34

2.



The applicant declares any amendment to the claims under Article 19 to be agreed with the examiner.

*The applicant is not required to answer questions 1 and 2 if the international preliminary examination is to be requested by the applicant.



Box No. VI CHECK LIST

The demand is accompanied by the following sheets, which are being submitted in accordance with Box No. IV, for the purposes of international preliminary examination:

- | | |
|--|--------|
| 1. translation of international application | sheets |
| 2. amendments under Article 34 | sheets |
| 3. copy (or, where required, translation) of amendments under Article 19 | sheets |
| 4. copy (or, where required, translation) of statement under Article 19 | sheets |
| 5. other (specify) | sheets |
| 6. other (specify) | sheets |

For International Preliminary Examining Authority use only

Article 17	Article 18
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<input type="checkbox"/>	<input type="checkbox"/>
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<input type="checkbox"/>	<input type="checkbox"/>

The demand is also accompanied by the item(s) marked below.

- | | |
|--|---|
| 1. <input checked="" type="checkbox"/> fee calculation sheet | 4. <input type="checkbox"/> statement explaining lack of signature |
| 2. <input type="checkbox"/> separate signed power of attorney | 5. <input type="checkbox"/> nucleotide and/or amino acid sequence listing in computer readable form |
| 3. <input type="checkbox"/> copy of general power of attorney; reference number, if any: | 6. <input checked="" type="checkbox"/> other (specify): Postcard: Associate Power of Atty. |

Box No. VII SIGNATURE OF APPLICANT, AGENT OR COMMON REPRESENTATIVE

Next to each signature, indicate the name of the person signing and the capacity in which the person signs (if such capacity is not obvious from reading the demand).


Diana Hamlet-Cox



PCT

FEE CALCULATION SHEET

Annex to the Demand for international preliminary examination

International application No. PCT/US00/24107	<div style="border: 1px solid black; padding: 5px;"> For International Preliminary Examining Authority use only IPEA/US 27 MAR 2001 Date stamp of the IPEA (27-03-01) </div>
Applicant's or agent's file reference PF-0738 PCT	
Applicant INCYTE GENOMICS, INC.	
Calculation of prescribed fees <div style="display: flex; justify-content: space-between; align-items: flex-end;"> <div style="width: 60%;"> 1. Preliminary examination fee </div> <div style="width: 35%; text-align: right;"> <div style="border: 1px solid black; padding: 2px 10px;">750.00</div> <div style="border: 1px solid black; padding: 2px 5px; margin-left: 5px;">P</div> </div> </div> <div style="display: flex; justify-content: space-between; align-items: flex-end; margin-top: 20px;"> <div style="width: 60%;"> 2. Handling fee <i>(Applicants from certain States are entitled to a reduction of 75% of the handling fee. Where the applicant is (or all applicants are) so entitled, the amount to be entered at H is 25% of the handling fee.)</i> </div> <div style="width: 35%; text-align: right;"> <div style="border: 1px solid black; padding: 2px 10px;">137.00</div> <div style="border: 1px solid black; padding: 2px 5px; margin-left: 5px;">H</div> </div> </div> <div style="display: flex; justify-content: space-between; align-items: flex-end; margin-top: 20px;"> <div style="width: 60%;"> 3. Total of prescribed fees Add the amounts entered at P and H and enter total in the TOTAL box </div> <div style="width: 35%; text-align: right;"> <div style="border: 1px solid black; padding: 2px 10px; margin-bottom: 5px;">887.00</div> <div style="border: 1px solid black; padding: 2px 10px;">TOTAL</div> </div> </div>	
Mode of Payment <div style="display: flex; justify-content: space-between;"> <div style="width: 45%;"> <input checked="" type="checkbox"/> authorization to charge deposit account with the IPEA (see below) </div> <div style="width: 45%;"> <input type="checkbox"/> cash </div> </div> <div style="display: flex; justify-content: space-between; margin-top: 10px;"> <div style="width: 45%;"> <input type="checkbox"/> </div> <div style="width: 45%;"> <input type="checkbox"/> </div> </div>	
Deposit Account Number	Date (day/month/year)
Signature	



HOME COPY

PCT

REQUEST

The undersigned requests that the present international application be processed according to the Patent Cooperation Treaty.

PCT/US 00/24107

International Application No.

31 AUG 2000 (5108.00)

PCT INTERNATIONAL APPLICATION R0/US

Name of receiving Office and PCT International Application

Applicant's or agent's file reference
(if desired) (12 characters maximum) PF-0733 PCT

Box No. I TITLE OF INVENTION
HUMAN HYDROLYTIC ENZYMES

Box No. II APPLICANT

Name and address: (Family name followed by given name for a legal entity, full official designation)
The address must include postal code and name of country. The country of the address indicated in this box is the applicant's State (that is, country) of residence if no State of residence is indicated below.

INCYTE GENOMICS, INC.
8150 Quince Road
Palo Alto, California 94304
United States of America

☐ This person is the inventor

Telephone No.
(650) 855-0555

Facsimile No.
(650) 845-4166

Teleprinter No.

State (that is, country) of nationality:
US

State (that is, country) of residence:
US

This person is applicant for the purposes of ☐ all designated States ☒ all designated States except the United States of America ☐ the United States of America only ☐ the States indicated in the Supplemental Box

Box No. III FURTHER APPLICANT(S) AND/OR (FURTHER) INVENTOR(S)

Name and address: (Family name followed by given name for a legal entity, full official designation)
The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.

YUE, Henry
826 Lois Avenue
Sunnyvale, California 94304
United States of America

This person is:

☐ applicant only

☒ applicant and inventor

☐ inventor only (If this check-box is marked, the applicant must be indicated in the preceding box.)

State (that is, country) of nationality:

State (that is, country) of residence:



Continuation of Box No. III FURTHER APPLICANTS AND OR (FURTHER) INVENTOR(S)

If none of the following sub-boxes is used, this sheet is not to be included in the request.

The address must include postal code and name of country. The country of the address indicated in this box is the applicant's State (that is, country) of residence, if no State of residence is indicated below.

230 Monroe Drive, #17
Mountain View, California 94049
United States of America

This person is

- ☐ applicant only
☒ applicant and inventor
☐ inventor only (If this check-box is marked, do not fill in below.)

State (that is, country) of nationality
US

State (that is, country) of residence
US

This person is applicant for the purposes of ☐ all designated States ☐ all designated States except the United States of America ☒ the United States of America only ☐ the States indicated in the Supplemental Box

Name and address (family name followed by given name, for a legal entity, full official designation). The address must include postal code and name of country. The country of the address indicated in this box is the applicant's State (that is, country) of residence, if no State of residence is indicated below.

TANG, Y. Tom
4230 Ranwick Court
San Jose, California 95118
United States of America

This person is

- ☐ applicant only
☒ applicant and inventor
☐ inventor only (If this check-box is marked, do not fill in below.)

State (that is, country) of nationality
CN

State (that is, country) of residence
US

This person is applicant for the purposes of ☐ all designated States ☐ all designated States except the United States of America ☒ the United States of America only ☐ the States indicated in the Supplemental Box

Name and address (family name followed by given name, for a legal entity, full official designation). The address must include postal code and name of country. The country of the address indicated in this box is the applicant's State (that is, country) of residence, if no State of residence is indicated below.

SAVIO, J. N. Maria
14244 Santiago Road
San Leandro, California 94577
United States of America

This person is

- ☐ applicant only
☒ applicant and inventor
☐ inventor only (If this check-box is marked, do not fill in below.)



Continuation of Box No. III - FURTHER APPLICANTS AND/OR (FURTHER) INVENTOR(S)

If name of the following sub-boxes is used, this sheet is not to be included in the request.

The address must include postal code and name of country. The country of the address indicated in this box is the applicant's State (that is, country) of residence if no State of residence is indicated below.

AZIMZAR, Inc.
2045 Rock Springs Drive
Hayward, California 94545
United States of America

This person is:

- ☐ applicant only
☒ applicant and inventor
☐ inventor only (If this check-box is marked, do not fill in below.)

State (that is, country) of nationality:
US

State (that is, country) of residence:
US

This person is applicant for the purposes of: ☐ all designated States ☐ all designated States except the United States of America ☒ the United States of America only ☐ the States indicated in the Supplemental Box

Name and address: (Family name followed by given name, for a legal entity, full official designation)
The address must include postal code and name of country. The country of the address indicated in this box is the applicant's State (that is, country) of residence if no State of residence is indicated below.

This person is:

- ☐ applicant only
☐ applicant and inventor
☐ inventor only (If this check-box is marked, do not fill in below.)

State (that is, country) of nationality:

State (that is, country) of residence:

This person is applicant for the purposes of: ☐ all designated States ☐ all designated States except the United States of America ☐ the United States of America only ☐ the States indicated in the Supplemental Box

Name and address: (Family name followed by given name, for a legal entity, full official designation)
The address must include postal code and name of country. The country of the address indicated in this box is the applicant's State (that is, country) of residence if no State of residence is indicated below.

This person is:

- ☐ applicant only
☐ applicant and inventor
☐ inventor only (If this check-box is marked, do not fill in below.)





Supplemental Box

If the Supplemental Box is not used, this sheet need not be included in the request.

If the space is insufficient, the applicant may file a separate sheet for each additional person, application, or State involved, and indicate the location of each such sheet in the Supplemental Box.

- if more than two persons are involved as applicants and/or inventors, in addition to the information required in Box No. II, write "Continuation of Box No. III" and indicate for each additional person the same type of information as required in Box No. III. Also indicate the office(s) of each such person, and the country or countries in which each such person is a resident or domiciled.
- (iii) if, in Box No. II or in any of the sub-boxes of Box No. III, the indication **"the States indicated in the Supplemental Box"** is checked, in such case, write "Continuation of Box No. II" or "Continuation of Box No. III" or "Continuation of Boxes No. II and No. III" (as the case may be), indicate the name of the applicant(s) involved and, next to each such name, the State(s) (and/or, where applicable, ARIPO, Eurasian, European or OAPI patent) for the purposes of which the named person is applicant.
- (iv) if, in Box No. II or in any of the sub-boxes of Box No. III, the inventor or the inventor/applicant is not inventor for the purposes of all designated States or for the purposes of the United States of America; in such case, write "Continuation of Box No. II" or "Continuation of Box No. III" or "Continuation of Boxes No. II and No. III" (as the case may be), indicate the name of the inventor(s) and, next to each such name, the State(s) (and/or, where applicable, ARIPO, Eurasian, European or OAPI patent) for the purposes of which the named person is inventor.
- (v) if, in addition to the agent(s) indicated in Box IV, there are further agents, in such case, write "Continuation of Box No. IV" and indicate for each further agent the same type of information as required in Box No. IV.
- (vi) if, in Box No. V, the name of any State (or OAPI) is accompanied by the indication **"patent of addition,"** or **"certificate of addition,"** or if, in Box No. V, the name of the United States of America is accompanied by an indication **"continuation"** or **"continuation-in-part"**; in such case, write "Continuation of Box No. V" and the name of each State involved (or OAPI), and after the name of each such State (or OAPI), the number of the parent title or parent application and the date of grant of the parent title or filing of the parent application.
- (vii) if, in Box No. VI, there are more than three earlier applications whose priority is claimed; in such case, write "Continuation of Box No. VI" and indicate for each additional earlier application the same type of information as required in Box No. VI.
- (viii) if, in Box No. VI, the earlier application is an ARIPO application; in such case, write "Continuation of Box No. VI", specify the number of the item corresponding to that earlier application and indicate at least one country party to the Paris Convention for the Protection of Industrial Property or one Member of the World Trade Organization for which that earlier application was filed.

2. If, with regard to the **precautionary designation statement** contained in Box No. V, the applicant wishes to exclude any State(s) from the scope of that statement; in such case, write "Designation(s) excluded from precautionary designation statement" and indicate the name or two-letter code of each State so excluded.

3. If the applicant claims, in respect of any designated Office, the benefits of provisions of the national law concerning **non-prejudicial disclosures** or **exceptions to lack of novelty**; in such case, write "Statement concerning non-prejudicial disclosures or exceptions to lack of novelty" and furnish that statement below.

Continuation of Box No. V

US 60/151,819 filed 01 September 1999 (01.09.99)



Box No. VI PRIORITY CLAIM

Enter date of filing of earlier application in the receiving Office
 Number of the earlier application in the receiving Office

☐ Further priority claim indicated in the Supplemental Box.

Where earlier application is:

national application of the receiving Office international application of the receiving Office international application of another Office

15 September 1999 60/151819
 15 09 99

115

item (3)

☒ The receiving Office is requested to prepare and transmit to the International Bureau a certified copy of the earlier application(s) *only if the earlier application was filed with the Office which for the purposes of the present international application is the receiving Office* identified above as item(s) (1)

* When the earlier application is an ARIPO application, it is mandatory to indicate in the Supplemental Box at least one country party to the Paris Convention for the Protection of Industrial Property for which that earlier application was filed (Rule 4.10² ARIPO). See Supplemental Box.

Box No. VII INTERNATIONAL SEARCHING AUTHORITY

Choice of International Searching Authority (ISA)

If two or more International Searching Authorities are competent to carry out the international search, indicate the Authority chosen; the number code may be used.

Request to use results of earlier search; reference to that search (if a national search has been carried out by or requested from the International Searching Authority)

Date (day month year)

Number

Country (or regional Office)

ISA/EP

Box No. VIII CHECK LIST: LANGUAGE OF FILING

This international application contains the following number of sheets:

request

6

description (excluding sequence listing part)

81

claims

5

abstract

1

drawings

0

sequence listing part of description

25

Total number of sheets

118

This international application is accompanied by the item(s) marked below:

1 ☒ fee calculation sheet2 ☐ separate signed power of attorney3 ☐ copy of general power of attorney; reference number, if any:4 ☐ statement explaining lack of signature5 ☐ priority document(s) identified in Box No. VI as item(s).6 ☐ translation of international application into (language):7 ☐ separate indications concerning deposited microorganism or other biological material8 ☒ nucleotide and/or amino acid sequence listing in computer readable form9 ☒ other (specify): Postcard; Transmittal

Figure of the drawings which should accompany the abstract:

n/a

Language of filing of the international application:

English

Box No. IX SIGNATURE OF APPLICANT OR AGENT

Signatures must be written in the name of the person signing and the capacity in which the person signs. If such signature is a facsimile, it must be certified as such.

[Signature]
 Agent for the Applicant

532 Rec'd PCT/PTC 31 AUG 2000

ISA



PATENT COOPERATION TREATY

PCT

From the INTERNATIONAL BUREAU

NOTIFICATION OF RECEIPT OF
RECORD COPY

(PCT Rule 24.2(a))

To:

HAMLET COX, Diana
Incyte Genomics, Inc.
3160 Porter Drive
Palo Alto, CA 94304
ETATS-UNIS D'AMERIQUE

Date of mailing (day/month/year) 19 October 2000 (19.10.00)	IMPORTANT NOTIFICATION
Applicant's or agent's file reference PF-0738 PCT	International application No. PCT/US00/24107

The applicant is hereby notified that the International Bureau has received the record copy of the international application as detailed below.

Name(s) of the applicant(s) and State(s) for which they are applicants:

INCYTE GENOMICS, INC. (for all designated States except US)
YUE, Henry et al (for US)

International filing date

31 August 2000 (31.08.00)

Priority date(s) claimed

01 September 1999 (01.09.99)

Date of receipt of the record copy
by the International Bureau

21 September 2000 (21.09.00)

List of designated Offices

AP : GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW

EA : AM, AZ, BY, KG, KZ, MD, RU, TJ, TM

EP : AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE

OA : BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG

National : AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MH, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW

ATTENTION

In addition, the applicant's attention is drawn to the information contained in the Annex to this notification.



the applicant's attention is drawn to the information contained in the Annex to this notification.



the applicant's attention is drawn to the information contained in the Annex to this notification.



the applicant's attention is drawn to the information contained in the Annex to this notification.

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Catherine Massetti



PATENT COOPERATION TREATY

FEB 26 2001

PCT

INTERNATIONAL SEARCHING AUTHORITY

INCYTE GENOMICS, INC.
Attn: HAMLET-COX, D.
3100 Porter Drive
Palo Alto, CA 94304
UNITED STATES OF AMERICA

INVITATION TO PAY ADDITIONAL FEES

(PCT Article 17(3)(a) and Rule 40.1)

Applicant's or agent's file reference PF-0738 PCT		Date of mailing (day month year) 21 02 2001
International application No. PCT/US 00/ 24107		PAYMENT DUE with 45 XXXX days from the date of mailing
Applicant INCYTE GENOMICS, INC.		International filing date (day month year) 31 08 2000

1. This International Searching Authority

considers that there are 12 (number of) inventions claimed in the international application covered by the claims indicated ~~XXXX~~ on the extra sheet:

and it considers that the international application does not comply with the requirements of unity of invention (Rules 13.1, 13.2 and 13.3) for the reasons indicated ~~XXXX~~ on the extra sheet:



International Searching Authority

Catherine Humbert



This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: 1-28 partially

Polypeptide of sequence SEQ ID NO:2 and encoding polynucleotide of SEQ ID NO:16, fragments, analogs and applications thereof.

2. Claims: 1-28 partially

Polypeptide of sequence SEQ ID NO:3 and encoding polynucleotide of SEQ ID NO:17, fragments, analogs and applications thereof.

3. Claims: 1-28 partially

Polypeptide of sequence SEQ ID NO:4 and encoding polynucleotide of SEQ ID NO:18, fragments, analogs and applications thereof.

4. Claims: 1-28 partially

Polypeptide of sequence SEQ ID NO:5 and encoding polynucleotide of SEQ ID NO:19, fragments, analogs and applications thereof.

5. Claims: 1-28 partially

Polypeptide of sequence SEQ ID NO:6 and encoding polynucleotide of SEQ ID NO:20, fragments, analogs and applications thereof.

6. Claims: 1-28 partially

Polypeptide of sequence SEQ ID NO:7 and encoding polynucleotide of SEQ ID NO:21, fragments, analogs and applications thereof.

Polypeptide of sequence SEQ ID NO:11 and encoding



applications thereof.

10. Claims: 1-28 partially

Polypeptide of sequence SEQ ID NO:12 and encoding polynucleotide of SEQ ID NO:26, fragments, analogs and applications thereof.

11. Claims: 1-28 partially

Polypeptide of sequence SEQ ID NO:13 and encoding polynucleotide of SEQ ID NO:27, fragments, analogs and applications thereof.

12. Claims: 1-28 partially

Polypeptide of sequence SEQ ID NO:14 and encoding polynucleotide of SEQ ID NO:28, fragments, analogs and applications thereof.

The present application relates human hydrolytic enzymes and uses thereof. Multiple human hydrolytic enzymes have been identified and characterized already in the prior art, as recognized by the applicant in the description, pages 1-4. In the light of the prior art the problem underlying the present application can be formulated as providing further human hydrolytic enzymes. The following solutions are proposed in the claims:

1. Human hydrolytic enzyme of sequence SEQ ID NO:2, as well as encoding polynucleotide of sequence SEQ ID NO:16.

2. Human hydrolytic enzyme of sequence SEQ ID NO:3, as well as encoding polynucleotide of sequence SEQ ID NO:17.

3. Human hydrolytic enzyme of sequence SEQ ID NO:4, as well as encoding polynucleotide of sequence SEQ ID NO:18.

polynucleotide of sequence SEQ ID NO:25.



10. Human hydrolytic enzyme of sequence SEQ ID NO:12, as well as encoding polynucleotide of sequence SEQ ID NO:26.

11. Human hydrolytic enzyme of sequence SEQ ID NO:13, as well as encoding polynucleotide of sequence SEQ ID NO:27.

12. Human hydrolytic enzyme of sequence SEQ ID NO:14, as well as encoding polynucleotide of sequence SEQ ID NO:28.

Given the essential difference between the sequences of the polypeptides and corresponding polynucleotides provided as solutions to problem, due to the fact that human hydrolytic enzymes have already been disclosed in the state of the art, and since in the light of the state of the art, no other technical feature could be distinguished as being new and common to the proposed problems and solutions, the ISA is of the opinion that there is no single inventive concept underlying the plurality of the claimed inventions in the present application, in the sense of Rule 13.1 PCT.

Consequently to this lack of unity, the different inventions are formulated as different subjects according to Article 17(3)(a) PCT.



FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 206

Continuation of Box 3.

Although claim 18 is directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the composition.

Further defect(s) under Article 17(2)(a):

Continuation of Box 3.

Claims Nos.: 20, 21, 23, 24

Present claims 20, 21, 23 and 24 relate to a compound defined by reference to a desirable characteristic or property, namely agonist or antagonist activity towards the polypeptide of claim 1. The claims cover all compounds having this characteristic or property, whereas the application does not provide support within the meaning of Article 6 PCT and/or disclosure within the meaning of Article 5 PCT for any specific example of such compounds. In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible. Consequently, no search has been carried out for claims 20, 21, 23 and 24.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.



Annex to Form PCT/ISA/206
 COMMUNICATION RELATING TO THE RESULTS
 OF THE PARTIAL INTERNATIONAL SEARCH

International Application No.

PCT/US 00/24107

- 1 The present communication is an Annex to the invitation to pay additional fees (Form PCT/ISA/206). It shows the extent of the international search results disclosed on the parts of the international application which relate to the invention first mentioned in claim Nos.
- 2 This communication is not the international search report which will be established according to Article 16 and rule 43 (see "Invitation to pay additional fees").
- 3 If the applicant does not pay any additional search fees, the information appearing in this communication will be limited and a search of the prior art will not be included in such an international search report.
- 4 If the applicant pays additional fees, the international search report will contain both the information appearing in this communication and the results of the international search on other parts of the international application for which such fees will have been paid.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication where appropriate of the relevant passages	Relevant to search
X	DATABASE EMBL 'Online! Entry AF015416. 26 August 1997 (1997-08-26) EVANS G.A. ET AL.: "95 kb from four overlapping human chromosome 11p15.5 cosmids" XP002159698 nucleotides 78880-80130 -----	11-15



INTERNATIONAL SEARCH REPORT

International Application No.
PCT/00/24107

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C12N15/55 C12N9/14 C12N1/21 A01K67/027 C07K16/40
C12Q1/68 A61K38/46 G01N33/68

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C12N A01K C07K C12Q G01N A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EMBL, EPO-Internal, WPI Data, BIOSIS, MEDLINE, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No
------------	--	----------------------

X	DATABASE EMBL [Online] Entry AF015416, 26 August 1997 (1997-08-26) EVANS G.A. ET AL.: "95 kb from four overlapping human chromosome 11p15.5 cosmids" XP002159698 nucleotides 78880-80130 -----	11-15
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☐ Further documents are listed in the continuation of box C.

☐ Patent family members are listed in annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"S" document member of the same patent family

Date of the actual completion of the international search

7 February 2001

Date of mailing of the international search report

1 0. 05. 01

Name and mailing address of the ISA

European Patent Office P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel (+31-70) 340-2040, Tx 31 651 epo nl.
Fax (+31-70) 340-3016

Authorized officer

MONTERO LOPEZ B.



INTERNATIONAL SEARCH REPORT

Intern. Application No

PCT/00/07560

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No
X	<p>FENNELLY: "MUCOSAL DNA VACCINE IMMUNIZATION AGAINST MEASLES WITH A HIGHLY ATTENUATED SHIGELLA FLEXNERI VECTOR" JOURNAL OF IMMUNOLOGY, vol. 162, February 1999 (1999-02), pages 1603-1610, XP002164285 cited in the application page 1604, paragraph 10 page 1605, paragraph 4 page 1606, paragraph 1 -page 1608, paragraph 2 page 1609, paragraph 2</p>	26,29, 31,33
Y	<p>KAREM K L ET AL: "PROTECTIVE IMMUNITY AGAINST HERPES SIMPLEX VIRUS (HSV) TYPE 1 FOLLOWING ORAL ADMINISTRATION OF RECOMBINANT SALMONELLA TYPHIMURIUMVACCINE STRAINS EXPRESSING HSV ANTIGENS" JOURNAL OF GENERAL VIROLOGY,GB,SOCIETY FOR GENERAL MICROBIOLOGY, READING, vol. 78, February 1997 (1997-02), pages 427-434, XP002910293 ISSN: 0022-1317 cited in the application page 427 abstract</p>	6
Y	<p>OKADA E ET AL: "Intranasal immunization of a DNA vaccine with IL - 12 - and granulocyte-macrophage colony-stimulating factor (GM-CSF)-expressing plasmids in liposomes induces strong mucosal and cell-mediated immune responses against HIV-1 antigens" JOURNAL OF IMMUNOLOGY,US,THE WILLIAMS AND WILKINS CO. BALTIMORE, vol. 159, no. 7, 1 October 1997 (1997-10-01), pages 3638-3647, XP002106202 ISSN: 0022-1767 page 3638 abstract</p>	16,17,20
A	<p>KUKLIN ET AL: "ROLE OF MUCOSAL IMMUNITY IN HERPES SIMPLEX VIRUS INFECTION" JOURNAL OF IMMUNOLOGY, vol. 160, 1998, pages 5998-6003, XP002164286 cited in the application page 5998 abstract</p>	

-/--



INTERNATIONAL SEARCH REPORT

Intern Application No

PCT/ 00/07560

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No
A	<p>PAGLIA ET AL: "GENE TRANSFER IN DENDRITIC CELLS, INDUCED BY ORAL DNA VACCINATION WITH SALMONELLA TYPHIMURIUM, RESULTS IN PROTECTIVE IMMUNITY AGAINST A MURINE FIBROSARCOMA"</p> <p>BLOOD, vol. 92, 1998, pages 3172-3176, XP002164287 cited in the application page 3172 abstract</p> <p>---</p>	
A	<p>DARJI A ET AL: "ORAL SOMATIC TRANSGENE VACCINATION USING ATTENUATED S. TYPHIMURIUM"</p> <p>CELL,US,CELL PRESS, CAMBRIDGE, NA, vol. 91, 12 December 1997 (1997-12-12), pages 765-775, XP002071290 ISSN: 0092-8674 cited in the application page 765, paragraph 5 -page 766, paragraph 6 page 772; figure 8</p> <p>---</p>	
A	<p>WO 98 44131 A (SIZEMORE DONATA R ;GROVE JASON C (US); SADOFF JERALD C (US); WALTE) 8 October 1998 (1998-10-08) cited in the application page 3, line 25 -page 5, line 19</p> <p>-----</p>	



FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

polynucleotide of SEQ ID NO:23, fragments, analogs and applications thereof.

9. Claims: 1-28 partially

Polypeptide of sequence SEQ ID NO:11 and encoding polynucleotide of SEQ ID NO:25, fragments, analogs and applications thereof.

10. Claims: 1-28 partially

Polypeptide of sequence SEQ ID NO:12 and encoding polynucleotide of SEQ ID NO:26, fragments, analogs and applications thereof.

11. Claims: 1-28 partially

Polypeptide of sequence SEQ ID NO:13 and encoding polynucleotide of SEQ ID NO:27, fragments, analogs and applications thereof.

12. Claims: 1-28 partially

Polypeptide of sequence SEQ ID NO:14 and encoding polynucleotide of SEQ ID NO:28, fragments, analogs and applications thereof.



FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Claims Nos.: 20, 21, 23, 24

Present claims 20, 21, 23 and 24 relate to a compound defined by reference to a desirable characteristic or property, namely agonist or antagonist activity towards the polypeptide of claim 1. The claims cover all compounds having this characteristic or property, whereas the application does not provide support within the meaning of Article 6 PCT and/or disclosure within the meaning of Article 5 PCT for any specific example of such compounds. In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible. Consequently, no search has been carried out for claims 20, 21, 23 and 24.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

